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NASA JSC Water Monitor System - City of Houston Field Demonstration

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CONTENTS

TABLES

FIGURES

SUMMARY

As an outgrowth of its involvement in water reclamation and water quality monitoring for both spacecraft and domestic applications, the NASA Lyndon B. Johnson Space Center conducted a project to develop and test an automated water quality monitoring system. The objective of this project was to develop a system that could perform water quality monitoring on-line and in real time, much as it would be done in a spacecraft. The design goal was to develop a system with the capability to determine conformance to future high effluent quality standards and to increase the potential for reclamation and reuse of water. The resulting system includes conventional commercial sensors, advanced organic sensors, NASA-developed micro-organism sensors, an automated sample collection system, and a data acquisition and display system that also has the capability to automatically control and standardize the sensors. This report describes the system and documents the development and initial field trial of the NASA water monitor system demonstration unit. Although testing of this unit was not sufficiently long or sufficiently comprehensive to adequately verify all system capabilities, fully automated operation over a sustained period with only routine manual adjustments was accomplished. Two major points were demonstrated: (1) the water monitor system has great potential in water monitoring and/or process control applications; and (2) it represents a vast improvement over conventional (grab sample) water monitoring techniques.

INTRODUCTION

Background

Since the beginning of manned space flight, NASA has been involved in the analysis, design, development, testing, and application of systems for water supply, water monitoring, and waste management. Throughout the earlier programs (Mercury through Apollo-Soyuz), it was possible to discard water after it had been used. However, long-duration multipersonnel missions or space stations will not be able to accept this penalty because a six-man crew could easily use 113 to 136 kilograms (250 to 300 pounds) of water per day, or 36 to 45 megagrams (40 to 50 tons) per year. Thus, reuse of water will be essential. In preparation for this future requirement, NASA has worked extensively in the development and demonstration of processes for the recovery of water from all potential sources (e.g., humidity condensate, used washwater, urine, and fecal flush water), the monitoring of spacecraft water for both chemical and biological contamination, and the reduction of waste to small-volume chemically and biologically inactive residues. The experience and facilities NASA has acquired in the course of this work are directly applicable to the development of compact and efficient water reclamation and monitoring systems for domestic use. Thus, as part of its continuing effort

to transfer advanced technology to the community and in recognition of the increasing freshwater shortage and the national concern about pollution, NASA has undertaken several programs in pollution monitoring and water reuse technology.

In 1971, NASA began a program to design a modular integrated utility system (MIUS).[1] The purpose of the MIUS program was to develop techniques for integrating electrical power generation, water processing, solid and liquid waste management, and environmental conditioning, using residual energy for utility functions. This program was conducted in cooperation with other government agencies such as the Department of Housing and Urban Development, the National Bureau of Standards, and the Environmental Protection Agency.

The NASA work on MIUS included reviewing applications for reuse of water, such as cooling towers, home recirculation systems involving nonpotable applications, and garden irrigation (ref. 1). However, it was discovered that no national standards exist to determine the acceptability of treated wastewater for reuse, particularly for human consumption. In addition, no monitoring system was available to provide adequate and timely verification of total water quality. These key issues, common to both NASA spacecraft and Earth-bound applications, led to the development of an automated water quality monitoring system to ensure the safety of treated wastewater.

A survey of in-house NASA developments relative to water monitoring revealed the following items with potential for near-term applications.

1. A water quality monitoring system at the NASA Lyndon B. Johnson Space Center (JSC) developed for spacecraft application that incorporated conventional sensors and a chemiluminescence biosensor (ref. 2)

2. A coliform detection concept using detection of metabolic gas evolution being developed on a laboratory basis at the NASA Langley Research Center (refs. 3 and 4)

3. Bioluminescence techniques sensitive to adenosine triphosphate (ATP) in living organisms being studied at the NASA Robert H. Goddard Space Flight Center

None of these developments were in an on-line configuration, nor did they provide any type of data acquisition and display system. The water monitor system (WMS) project begun at JSC was designed to integrate these developments into an on-line system that could provide a complete water quality overview.

A phased development program to build the WMS was implemented. The phases were as follows:

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[1]Christopher C. Kraft, "Illustration of the Application of NASA Technology to Housing Problems." NASA Report on Housing Development, Vol. 2, NASA Lyndon B. Johnson Space Center, 1972.

Phase I: Assembly and testing of a breadboard system in the MIUS Integrated Systems Test (MIST) laboratory at JSC

Phase II: Assembly and testing of a field demonstration system in the MIST laboratory

Phase III: System demonstration in a community wastewater treatment facility

Phase I was concluded in February 1975, and the results are described in reference 5. This report documents Phases II and III, which were completed in May 1977.

Program Objective

Previous water quality testing in the MIST facility, as in most community treatment plants, was done primarily on a laboratory basis and often required 1 to 2 weeks to get laboratory results. Even under the best conditions, the determination of some parameters, such as biological oxygen demand (BOD) and Escherichia coli (fecal coliform), required 4 to 5 days. The objective of the WMS was to perform water quality monitoring on-line and in real time, much as it would be done in a spacecraft. The design goal was to develop a system with the capability to determine conformance to future high effluent quality standards and to increase the potential for reclamation and reuse of water.

The following program objectives were established.

1. To develop an automated WMS for wastewater treatment system effluent monitoring

2. To focus and accelerate development of real-time micro-organism sensor technology transfer efforts within NASA

3. To demonstrate feasibility and reliability by correlating data with standard laboratory techniques

4. To develop the system to a field demonstration configuration and demonstrate it to municipalities

5. To develop model specifications

6. To publish and distribute the results to federal, state, and local agencies

Scope

The NASA approach to the development of the WMS was to place emphasis on the NASA biological sensors and the total WMS concept. Performance of the biosensors was of special interest because they were in the developmental

stage. Although NASA appreciated the fact that selection of commercial sensors was an important task, system development time and program dollars were not of a scope to permit extensive testing of the wide range of candidate equipment for each parameter. Therefore, some sensors installed in the WMS performed better than others and their ultimate success (or failure) in the WMS application was not a NASA "guaranteed" condition. Additionally, installation in the WMS was not to be construed as an endorsement by NASA; it was the overall system approach to on-line monitoring that was considered to be of importance.

The Phase II effort, consisting of the development, assembly, and initial checkout of the field demonstration unit, was begun in March 1975 and completed in February 1977. Phase III, field testing in a municipal wastewater treatment facility, occurred during April and May 1977 at the Southwest Wastewater Treatment Plant (SWTP) in Houston, Texas. A block diagram of these activities is shown in figure 1. The tasks were performed under contract NAS 9-15060 with the Boeing Company, Houston, Texas.

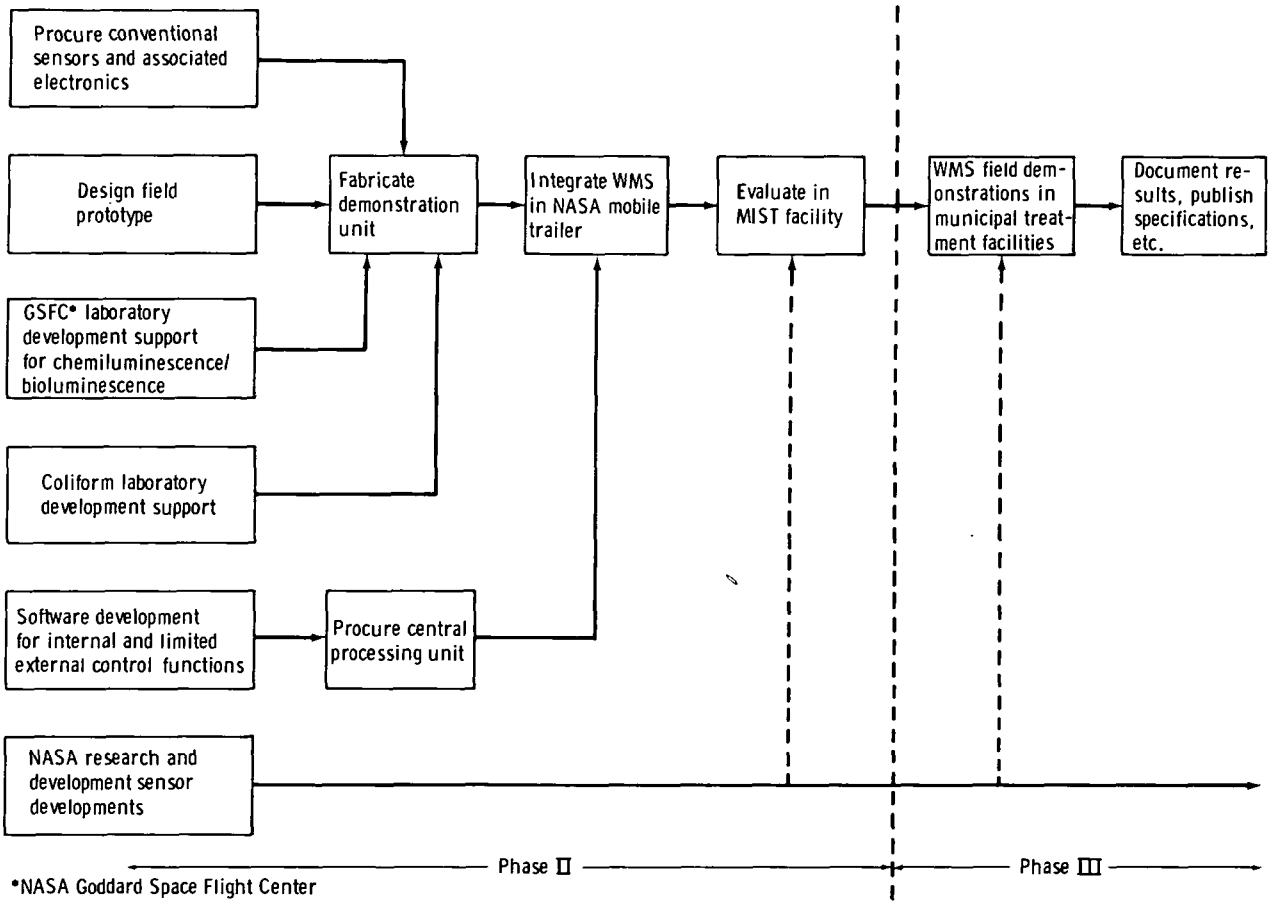


Figure 1.- Block diagram of the WMS project: Phases II and III.

The authors wish to express their appreciation to Nelson Milder, NASA Headquarters; S. K. Duncan, Richard Graves, Joy Robertson, and Gerald Taylor of JSC; Richard Brooks, Leo Chavez, Preston Dill, Art Linton, Vince Minnaert, Gene Moore, Robert Nuss, Jerry Poel, and Richard Thomas of The Boeing Company, Houston, Texas; Grace L. Picciolo and Emmett Chappelle of the NASA Goddard Space Flight Center; Judd Wilkins, NASA Langley Research Center; and personnel of Northrop Services, Incorporated, Houston, Texas.

In compliance with NASA publication policy, the original units of measure have been converted to the equivalent value in the Système International d'Unités (SI). As an aid to the reader, the SI units are written first and the original units are written parenthetically thereafter.

WATER MONITOR SYSTEM DESCRIPTION

The WMS is installed in a 9-meter (30-foot) air-conditioned mobile trailer that houses a sample conditioning/distribution system, sensors, and two data acquisition systems. The system can integrate a maximum of 40 parameters, which are scanned once each minute. Of the 40 channels, 32 are now in use: 5 for biological measurements, 18 for conventional sensors, and 9 for system pressures to provide data on the sample conditioning/distribution system. The WMS design would also allow remote devices to be connected by telephone through leased lines or dial-up modems. A description of the major system elements follows. Further information is available in reference 6.

Sample Conditioning/Distribution System

The sample conditioning/distribution system consists of the lines, valves, pumps, and filtration units that provide the sensors with the required flow rate and pressure from a predetermined source. It was designed to reduce the susceptibility of the sensors to contamination. As the schematic in figure 2 shows, the system is capable of "slipstream" intake from six different locations, such as the various stages of processing in a wastewater treatment plant. The plumbing is configured so that five channels, alternating at predetermined intervals provide water to one set of sensors. These channels are called "multipoint" sources. The sixth channel continuously supplies a second set of sensors with a sample of the plant's effluent (output). Both streams of sample water are directed so that they are available to the sensor packages in either a filtered or an unfiltered state. To avoid frequent maintenance, the filters are automatically backwashed every 15 seconds. A conductivity meter, placed upstream of the sensors, serves as a loss of fluid flow indicator and automatically shuts down the sensors when flow stops.

Sensors

The WMS includes 18 commercial sensors, which monitor 14 conventional chemical and physical water quality parameters, and 3 types of NASA-developed sensors, which monitor the microbiological quality of the water. All the sensors are suitable for wastewater, potable water, or natural water

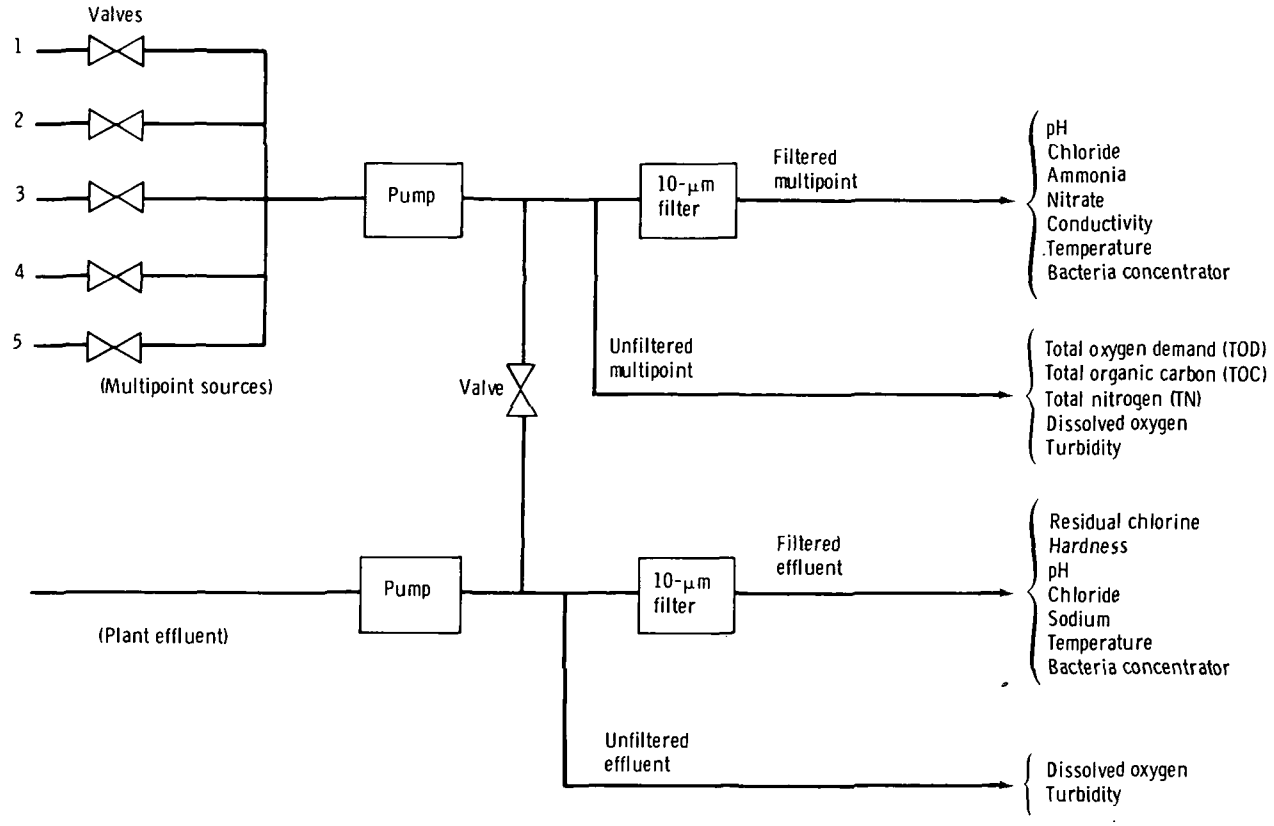


Figure 2.- Sample conditioning and distribution system.

applications. They were selected or developed on the basis of their applicability to on-line, near-real-time monitoring with the output being used by a computer to evaluate water quality and/or control unit processes. Six of these commercial sensors were procured and installed in the WMS during the Phase II development program. These sensors provided a more comprehensive water quality overview and replaced some of the original sensors that were either no longer giving satisfactory service or no longer representative of an adequate state of the art.

The biosensors detect and quantify bacterial cells, both living and dead. Ten biosensors are incorporated in the WMS: one chemiluminescence sensor, one bioluminescence sensor, and eight coliform detectors. Details of the early laboratory development and operation of these sensors are available in references 7 and 8. All three of the NASA-developed sensors were improved and placed on-line in the WMS during Phase II.

All the biosensors have access to a sample concentration system (ref. 9) that filters out bacteria while processing the sample fluid. This sytem provides better than 80-percent particle recovery at concentrations of up to 600-fold (5-fold to 20-fold per minute), using 1 square meter (10 square feet) of filter area. A multiple backwash procedure is used for optimum recovery.

A complete list of sensors is given in table I. New sensors are designated as Phase II replacement or addition. Descriptions of the biosensors are given in the following sections. Descriptions of the commercial sensors are given in appendix A.

Luminescence sensors.- Chemiluminescence is based on the precise measurement of light emitted by the catalytic action of bacterial porphyrins on a luminol/hydrogen peroxide mixture. The bacterial porphyrins are extracted by rupturing the cell wall with sodium hydroxide. Light emission is virtually instantaneous on addition of the aqueous luminol reagent. Because light emission is based on the presence of bacterial metal porphyrins that remain essentially unchanged when cells die, total cell quantities are measured. Although it is not a measurement of viability, the luminol assay is inexpensive ($0.20 per day) and gives a rapid reliable measure of general micro-organism population. Sensor response is linear above 10^4 cells/ml. A flow diagram of the sensor is shown in figure 3.

Bioluminescence is based on light emitted by the reaction of a firefly extract with ATP from bacterial cells. The bacterial ATP is extracted by rupturing the cell wall with nitric acid; it is then mixed with the enzyme in view of a light detector. The light intensity peaks within 1 second, followed by a logarithmic decay. The enzyme, luciferase, is pumped from a refrigerator located adjacent to the biosensor cabinet in order to maintain a high level of enzyme activity for at least 24 hours. The cost of the enzyme ($0.40 to $4.00 per milliliter, depending on purity) precludes a continuous-flow operation; consequently, a minimum volume (0.2 milliliter) is injected by valve actuation when a measurement is desired. ATP has a short survival time after cell death; thus, the reaction is considered specific for viable cells. The response of the sensor is linear above 10^6 cells/ml. A flow diagram is given in figure 4.

The major components of the luminescence sensors are identical. They include a fluid storage and transport system, made up of peristaltic pumps and associated Teflon valves and 0.8- and 1.6-millimeter tubing; a light detection photomultiplier, located so that its light-sensitive surface views the spiral-wound glass reaction cell where the sample and reagent are mixed; a manual/automatic control switch panel and instrumentation; and a strip-chart recorder.

Either concentrated or unconcentrated samples may be selected from the continuous sample supplied by the WMS. The sensors have a response time of 10 minutes. If sample concentration is necessary, readings can be made approximately every 30 to 45 minutes. Routine operation and calibration is computer controlled. Manual control is used for periodic maintenance. The output signal is locally displayed on the photometer and recorder and is also routed to the computer. The computer calculates bacterial concentrations, displays current results on a cathode-ray tube (CRT), and stores the raw data for future analysis and reporting.

Figure 5 is a photograph of the automated luminescence systems installed in the WMS. A standard 48-centimeter (19-inch) rack houses both sensors and the bacteria concentrator system.

TABLE I.- WMS SENSORS

Analysis	Sensor	Manufacturer	Sample source	Principle (a)
Ammonia	Photometer	Delta Scientific	Multipoint 10-μm filtered	Wet chemistry - electrochemical
Chloride[b]	Electrode	Great Lakes Instruments	Multipoint 10-μm filtered	Electrochemical
Conductivity	Impedance bridge	Beckman Instruments, Inc.	Multipoint 10-μm filtered	Ionic activity $\propto \frac{1}{\text{electrical resistance}}$
Dissolved oxygen	Electrode	Delta Scientific	Multipoint Unfiltered	Electrochemical oxidation-reduction
Dissolved oxygen	Electrode	Honeywell, Inc.	Effluent Unfiltered	Electrochemical oxidation-reduction
Hardness	Electrodes	Orion Research, Inc.	Effluent 10-μm filtered	Wet chemistry - electrochemical
Nitrate/nitrite	Photometer	Delta Scientific	Multipoint 10-μm filtered	Wet chemistry - electrochemical
pH[c]	Electrode	Great Lakes Instruments	Multipoint 10-μm filtered	Electrochemical
pH	Electrode	Orion Research, Inc.	Effluent 10-μm filtered	Electrochemical
Sodium	Electrode	Beckman Instruments, Inc.	Effluent 10-μm filtered	Electrochemical
Temperature	Resistance thermal detector	Action Pac	Multipoint and effluent 10-μm filtered	Thermoelectrical
TOC[b]	Infrared detector	Astro Ecology Corp.	Multipoint Unfiltered	$C + O_2 \xrightarrow[\text{pH 3}]{1123.15\ K\ (850^\circ\ C)} CO_2$ (infrared absorption)
TOD[b]	Fuel cell	Astro Ecology Corp.	Multipoint Unfiltered	Oxygen consumed in high-temperature (1123.15 K (850° C)) oxidation of organic materials
Total nitrogen[c]	Fuel cell	IBC/ Berkeley	Multipoint Unfiltered	$N + 1/2\ O_2 \xrightarrow[\text{Catalyst}]{1123.15\ K\ (850^\circ\ C)} NO$
Turbidity[c]	Photocell	Sigrist	Unfiltered	Nephelometric-light scatter by particles in suspension
Turbidity	Photocell	Honeywell, Inc.	Unfiltered	Nephelometric-light scatter by particles in suspension
Bioluminescence/ bacteria (living)[c]	Photomultiplier tube	NASA/ Boeing	Multipoint 10-μm filtered	ATP + firefly enzyme $\xrightarrow{Mg^{++}}$ products + light
Chemiluminescence/ bacteria (living and dead)[c]	Photomultiplier tube	NASA/ Boeing	Multipoint 10-μm filtered	Luminol + $H_2O_2 \xrightarrow{\text{Porphyrins}}$ products + light
Coliform (total or fecal)[c]	H_2 electrode	NASA/ Boeing	Multipoint 10-μm filtered	Coliform + nutrient $\xrightarrow[317.65\ K\ (44.5^\circ\ C)]{308.15\ K\ (35^\circ\ C)} H_2$

[a] C = carbon, O = oxygen, N = nitrogen, Mg = magnesium, H = hydrogen.
[b] Phase II replacement.
[c] Phase II addition.

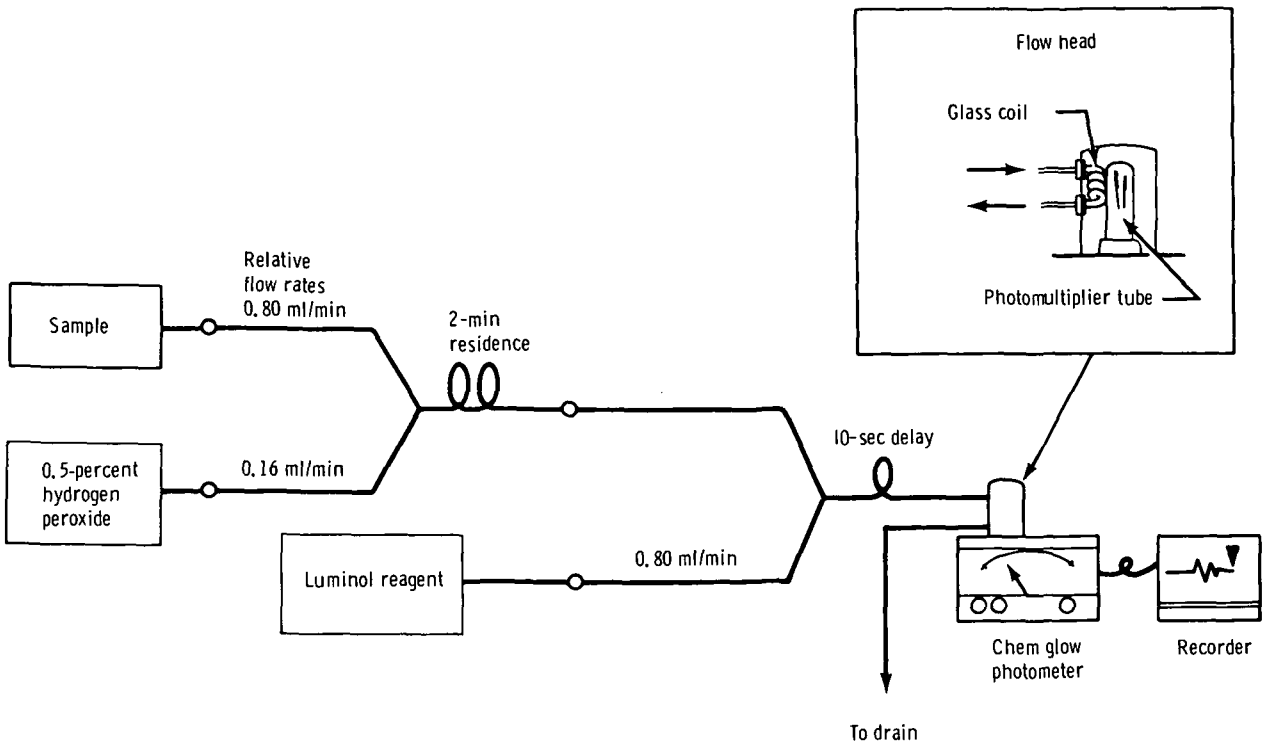


Figure 3.- Flow diagram of chemiluminescence sensor.

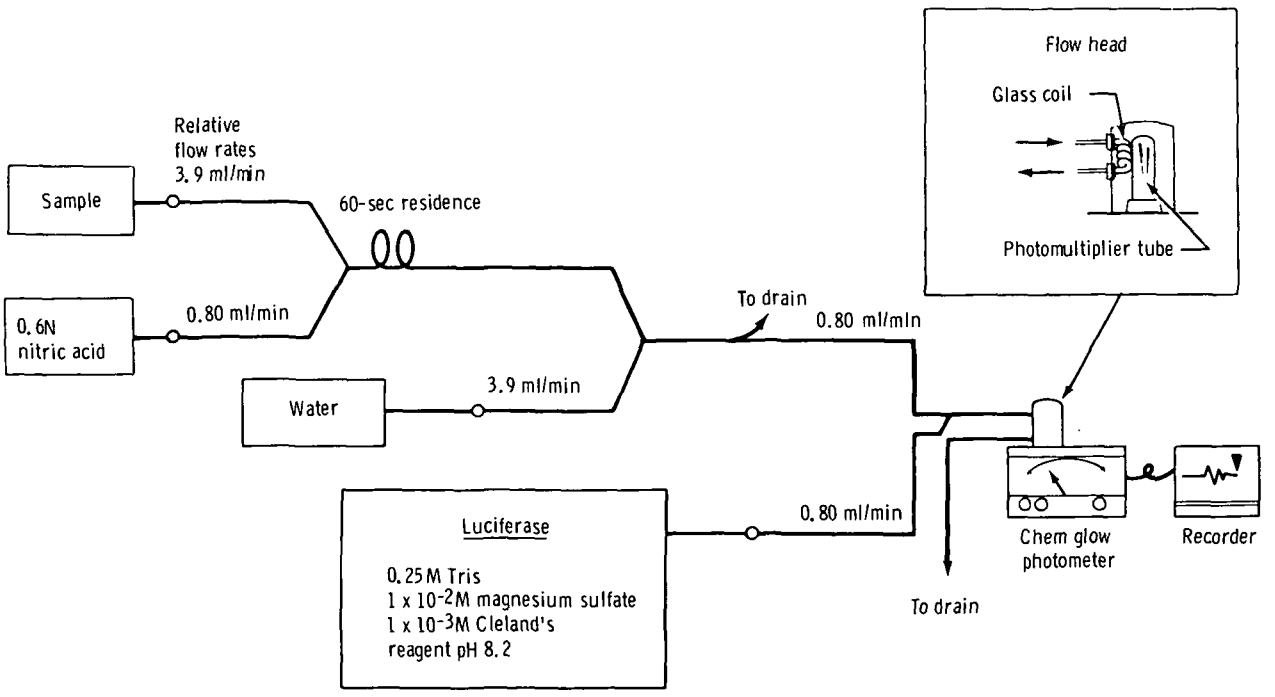


Figure 4.- Flow diagram of bioluminescence sensor.

Coliform detector.- The coliform detector sensors quantify organisms of the coliform group. Water samples are dispensed into a nutrient medium selective for coliform organisms, Lauryl Tryptose broth; if coliform are present, hydrogen is produced as a byproduct of their metabolism. Differentiation within the coliform group is possible since incubation at 308.15 K (35° C) results in evolution of hydrogen from the total coliform present, whereas at 317.65 K (44.5° C) only fecal coliform produce hydrogen. A platinum/calomel electrode inserted in the nutrient medium senses the gas. The time required for the production of detectable amounts of hydrogen is dependent on the concentration of coliform organisms in the sample. Hydrogen evolution is linear above 1 cell per sample with a response time of 0 to 12 hours. Response time can be lessened by concentrating the samples.

The detector is made up of four subsystems: (1) manual and automatic control switch panel, (2) eight incubator cells, (3) fluids storage and transfer equipment, and (4) instrumentation. The entire assembly is packaged in a standard 48-centimeter (19-inch) cabinet as shown in the photograph in figure 6. Four sensors are used for total coliform and four for fecal coliform determinations, with one of the eight being run as a blank. Automatic control of cleanup, nutrient fill, and incubation temperatures is provided by the computer. However, at the present state of development, the sample is manually injected to prevent cell contamination.

The eight incubator cells are serially connected by three-way Teflon valves to a common fluid fill and drain manifold. The cells are 25- by 150-millimeter glass test tubes. The tops of the cells are fitted with silicone stoppers molded for two 1.6-millimeter fill-drain and vent tubes, a 12-millimeter combination electrode, and a 0.2°-division mercury thermometer. Each of the cell tubes is immersed in its own mineral oil bath, which can be individually controlled at 308.15 K (35° C) (total coliform) or 317.65 K (44.5° C) (fecal coliform) and a bacteriostat temperature of 358.15 K (85° C). The bath is heated with electrical resistance heaters, and temperatures are maintained with solid-state proportional controllers. The fluids storage and handling section includes valves, a peristaltic pump, reagent storage containers, regulated facility air, and hot demineralized water. Air supplies are filtered to 0.45 micrometer and washed in 0.1N nitric acid. Organism growth is monitored with one combination electrode per cell. The electrode signal is conditioned with high-impedance amplification and multiplexed to the computer for visual display and engineering unit printout.

Data Acquisition Systems

Two separate data collection and display systems are on-line in the WMS: a computer and a hardwired fixed-format device (Phase I data acquisition system (DAS)). Lack of report generation capability in the Phase I DAS and delays in obtaining processed data from a central data processing system resulted in time-consuming manual compilation and analysis of substantial amounts of data. In the Phase I Test Report, the test team recommended that automated data logging, evaluation (including statistical compilations), and report generation be provided in future systems. Automated standardization (calibration) of sensors was also recommended bacause of the amount of time

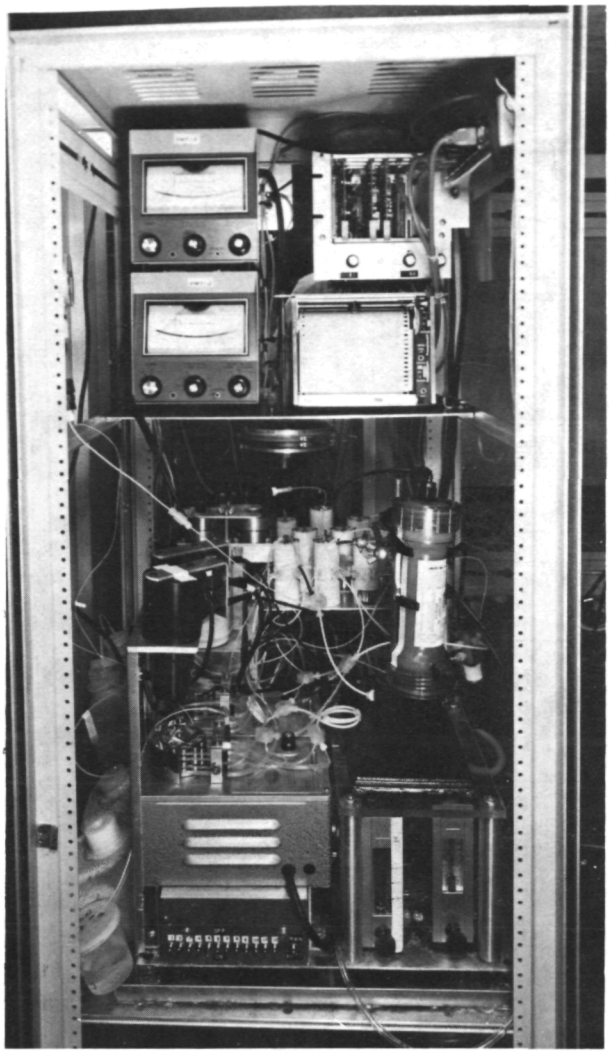


Figure 5.- Automated luminescence sensors and bacteria concentrator.

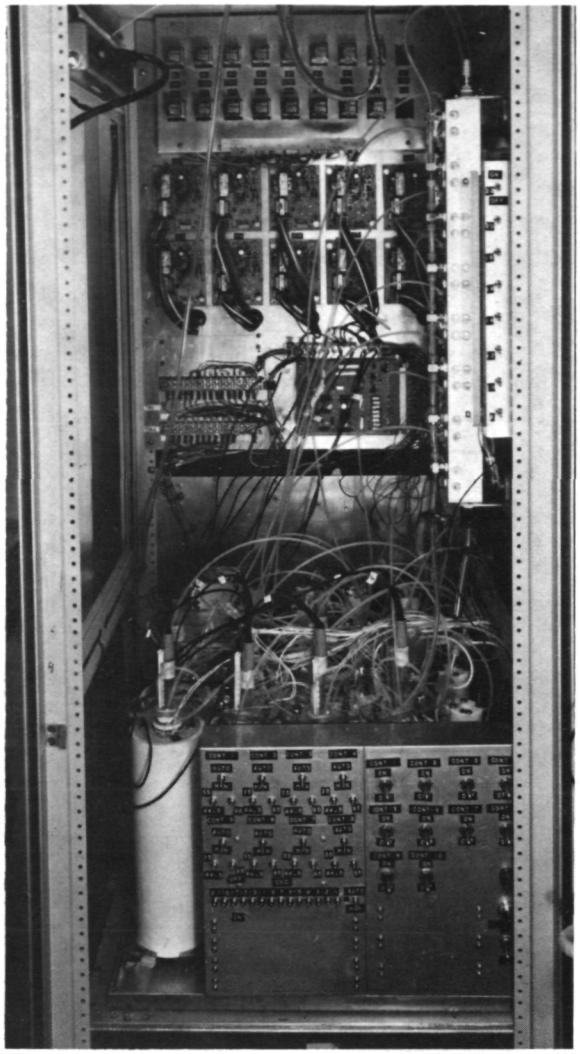


Figure 6.- Automated coliform detector.

required for manual standardization of the large number of sensors in the system. Therefore, the computerized DAS was incorporated during Phase II. Figure 7 is a flow diagram of the computer functions and the interface with the Phase I DAS.

Phase I DAS.- The Phase I DAS is a manually operated system. It can be used with the computer or by itself. Alone, however, it has limited capability for growth and data processing applications. The system consists of an analog section, a digital section, two cassette decks, and appropriate power supplies.

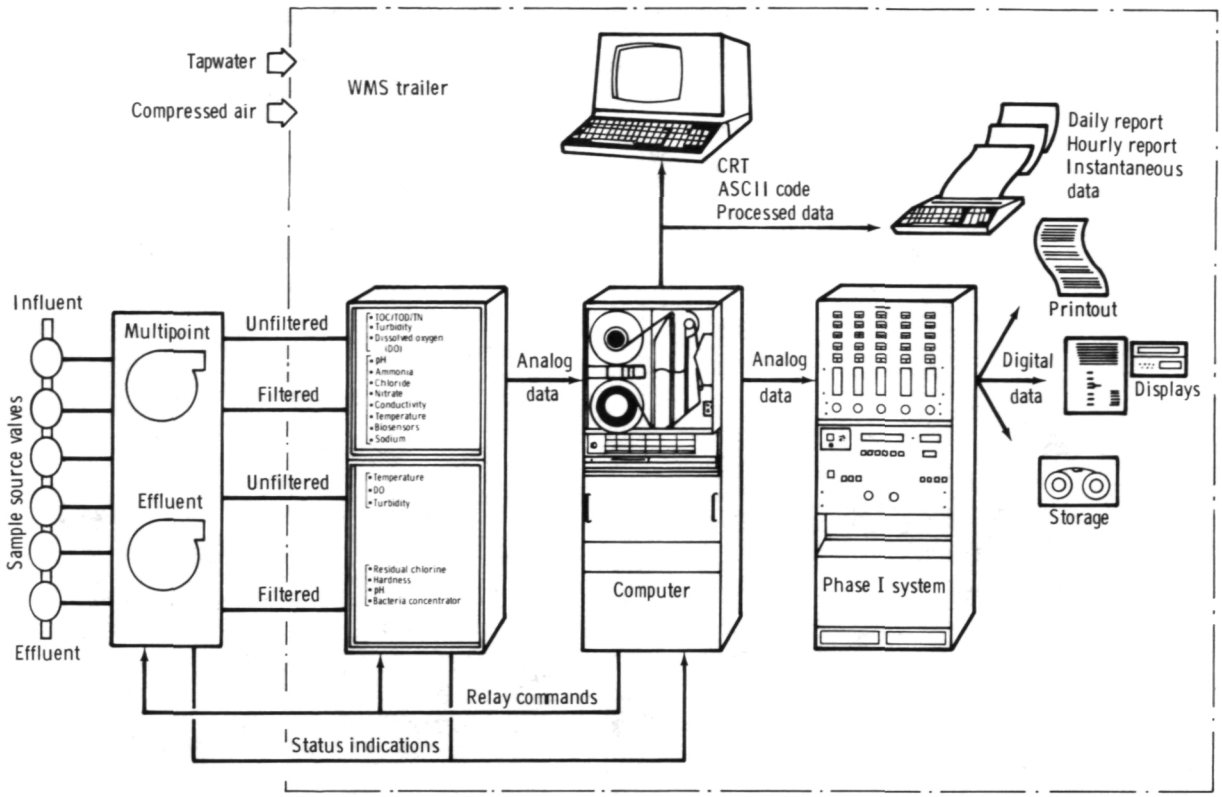


Figure 7.- Data flow diagram.

The system can accept as many as 25 (0 to 5 volt) analog input signals. Three scan patterns and two sampling speeds are provided and can be automatically or manually selected. In the normal mode, the signals are digitized and arrayed into 75 time slots during each 15-minute data period. The accumulated data are then dumped onto a cassette tape during a 6-second "on" period. This design permits storage times in excess of 24 hours on each 60-minute cassette tape. A second cassette deck permits playback and display of historical data without interrupting real-time data monitoring.

The audio recorders used during Phase I were found to have a high bit-error rate. In Phase II, these recorders were replaced with commercial digital incremental recorders. An engineering unit converter/printer was also added to the system during Phase II to provide the capability for hard-copy reports of data at a selectable printout rate.

Phase II computerized DAS.- The computer system is based on two Data General Corporation NOVA minicomputers--one to acquire data and automatically standardize the commercial sensors and the other to generate reports and control the biosensors. The data acquisition computer system was purchased from Monitor Labs, Inc., San Diego, California, and is referred to as ADAM (air data acquisition and monitoring). ADAM employs a vendor-proprietary software

package programed in assembly code. Some modifications were made to the standard software to accommodate additional valve status indications and data transfer requirements. The report generation computer system, developed by the Boeing Company, Houston, Texas, provides additional reporting and control capability not available in ADAM. It is referred to as EVE (environmental verification and evaluation) and was developed around the Data General Corporation Real-Time Disk Operating System, using Fortran IV for all program instructions.

Operator interface with the WMS is provided by a separate communication device for each computer: a thermal printer and keyboard for ADAM and a CRT data screen and keyboard for EVE. Instrument status and current and historical data are monitored on the CRT. Hard copies of any of these can be printed on a Versatec line printer attached to EVE.

The ADAM system scans all commercial sensors once each minute and transmits the readings to EVE. The data are processed by EVE, displayed on the CRT, and simultaneously stored on disk files. Biosensor data are fed directly to EVE for processing and storage. Using the data on the disk, EVE can generate reports of hourly averages for the current day or any day within a 3-month period, daily reports, and biosensor reports. Instantaneous reports provide the previous 1-minute value and the previous 15-minute, 30-minute, and hourly averages, as well as the cumulative averages for each of the available 40 channels. The historical data reports provide the hourly averages for each hour of any day on record. Daily reports give the daily averages and the instantaneous and hourly peak values and the time of day each occurred. The biosensor reports show voltages recorded by the coliform detectors and the luminescence sensors and the cell count for living and dead bacteria.

Figure 8 is a photograph of the two data acquisition systems installed in the WMS trailer. More detailed information on the Phase II system can be found in reference 10.

TEST SUPPORT

The MIST wastewater treatment facility, the Houston SWTP, and the laboratory analyses performed for comparison to WMS values are described in this section.

MIST Wastewater Treatment Facility

The MIST wastewater treatment facility includes a wastewater storage and hydrosieve-screen unit for the control of large suspended solids, a rotating biological disk, and a packaged physical/chemical treatment system. Additional advanced technology systems are available and can be operated in series or in parallel to provide a maximum degree of experimentation. Wastewater is pumped to the system from a regular JSC sewage line.

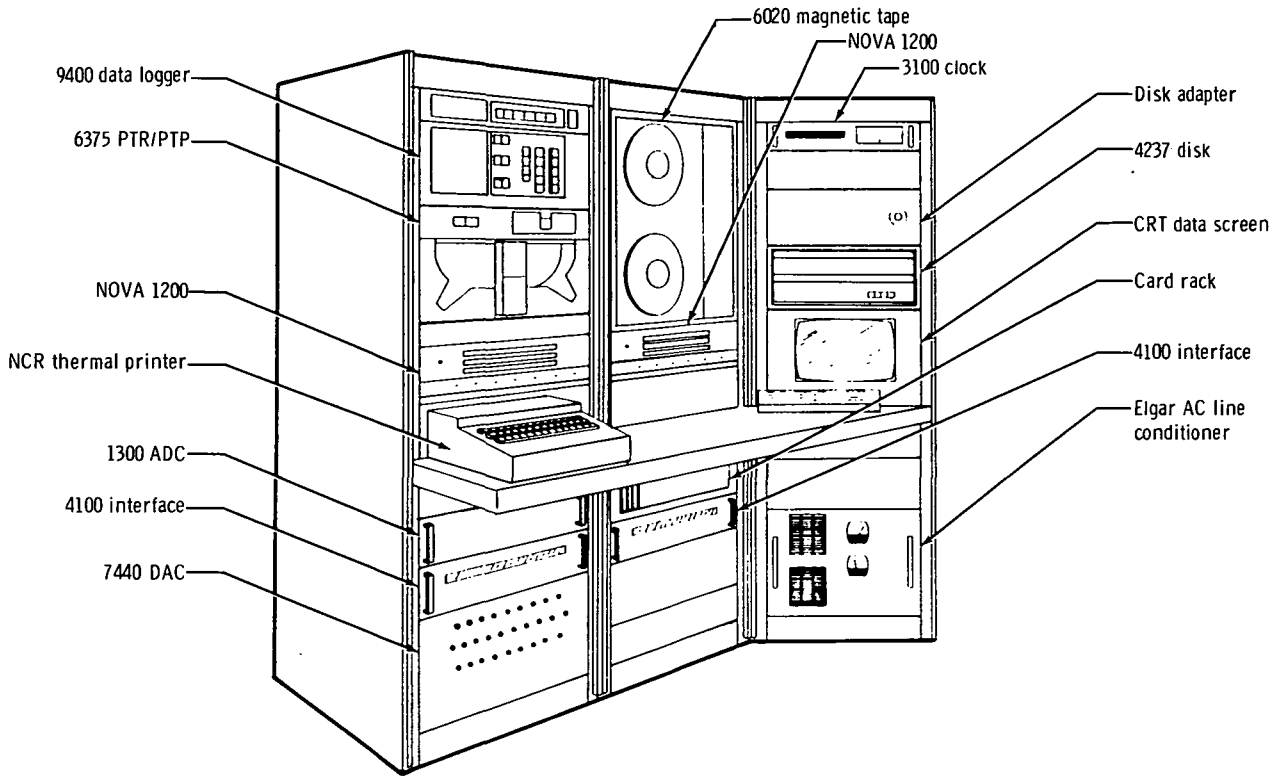


Figure 8.- WMS data acquisition systems.

The biological reactor is designed to permit the growth of micro-organisms on a rotating solid surface that brings them into contact with dissolved biodegradable organic pollutants and oxygen. The micro-organisms extract the organic pollutants from the wastewater and use them as their source of energy.

As a result, the dissolved materials are partially converted to biomass, a suspended solid, which is separated from the wastewater flow by sedimentation. The physical/chemical treatment system consists of unit processes designed to remove dissolved inorganic pollutants, such as hardness agents and phosphorous, as well as suspended and colloidal solids and color. Chlorine is used as the primary disinfectant.

The WMS used pumps and plastic tubing to draw process water from selected sampling points in the wastewater process to the trailer. External power was supplied by the MIST electrical system.

Houston Southwest Wastewater Treatment Facility

The SWTP in Houston is an activated sludge plant with a hydraulic design capacity of 340 65 m^3/day (90 million gallons per day (MGD)). The plant was

constructed in 1954 and enlarged in 1959, 1965, and 1969. It currently processes domestic and industrial waste at a flow rate of 0.095 to 0.114 m^3/day (25 to 30 MGD).

Raw sewage is pumped directly to aeration tanks and mixed with activated sludge. The plant has six aerated tanks with a total volume of 37 850 cubic meters (10 million gallons). Air is provided to the tanks by a series of compressors at the rate of 1133 to 1274 m^3/min (40 000 to 45 000 ft^3/min) (48 cubic meters per 0.45 kilogram (1700 cubic feet per pound) of BOD removed). During normal operation, half the tanks contain mixed liquor and half contain returned sludge. The solids concentration of the mixed liquor is approximately half that of the returned sludge.

Mixed liquor flows to six clarifiers for settling during a 2-hour detention time. About 1 million gallons of sludge are removed each day from the return sludge aeration tanks, and the rest is returned to the system.

Chlorination is used for disinfection. Clarifier effluent flows into a mixing tank where a 5-percent sodium hypochlorite solution is added through a manually controlled valve. An average of 523 kg/day (1152 lb/day) of chlorine was used in May 1977.

The plant's state permit specified the following standards for the effluent.

1. Residual chlorine no less than 1 ppm at any time

2. Monthly average BOD no greater than 20 ppm

To monitor the effluent, plant operators collect a grab sample just upstream of the weir every 2 hours. The samples from each 24-hour period are combined and taken to a laboratory for analysis. For comparison with the WMS, more frequent discrete samples were taken.

Sample points are indicated on the diagram of the plant in figure 9. Water was pumped from these points through polyethylene pipes. External electrical power was supplied to the WMS.

Laboratory Analysis Methods

Samples of water being monitored by the WMS were taken periodically for classical laboratory analyses. Results were used for comparison with WMS readings. Table II describes the laboratory analysis techniques used by the City of Houston's Quality Control Laboratories and by the JSC Environmental Health Services Laboratory in determinations reported under Phase III Field Testing and Results.

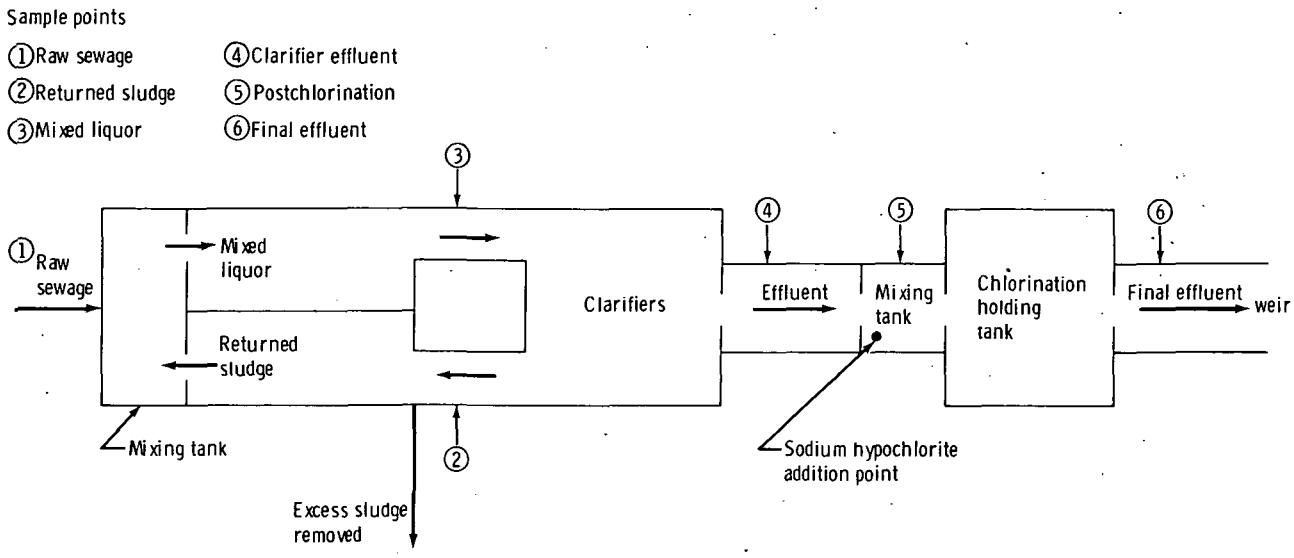


Figure 9.- Block diagram of City of Houston Southwest Wastewater Treatment Plant.

ASSEMBLY AND CHECKOUT: PHASE II

Assembly and checkout of the field demonstration unit during Phase II of the WMS project occurred at the MIST facility at JSC. The major tasks accomplished were as follows.

1. Design and assembly of the NASA biological detection systems

2. Procurement of new conventional sensors to upgrade the breadboard system

3. Modification of the system to eliminate operational problems discovered in Phase I

4. Upgrading of the DAS to provide automatic standardization of sensors and real-time data display capabilities

5. Integration of the system and installation in a NASA mobile trailer

6. Checkout testing of the system at the MIST facility at JSC

Primary emphasis during this period was on development and calibration of the NASA biosensors. Details of the development process are summarized in appendix B. Calibration results and test data are discussed in the following paragraphs.

TABLE II.- LABORATORY ANALYSIS TECHNIQUES

Analysis	Procedure	Laboratory (a)	Reference	Sensitivity, mg/liter
Ammonia	Orion specific ion electrode	1	-	0.03
BOD_5	Membrane electrode calibrated against Winkler method	1	11, p. 484	1
COD	Dichromate reflux	1	11, p. 495	10
Chloride	Titration - $HgNO_3$	1	11, p. 97	1
Hardness	Titration - Na_2EDTA	1	11, p. 179	1
Nitrate/nitrite	Aluminum reduction and Nessler's	1	11, p. 226	.1
pH	Single-junction glass versus calomel electrode	1	11, p. 500	(b)
Residual chlorine	Amperometric titration	1	11, p. 112	1
Total suspended solids	Filtration onto glass fiber filters	1	11, p. 539	10
Fecal coliform	Standard plate count	1 and 2	11, p. 660	-
Total coliform	Standard plate count	1 and 2	11, p. 660	-
Total bacteria	Standard plate count	1 and 2	11, p. 660	-

[a]Laboratory 1, City of Houston Quality Control Laboratories; Laboratory 2, JSC Environmental Health Services Laboratory.
[b]0.1 unit.

A chemiluminescence system calibration was performed using the five most prevalent bacteria species found in JSC wastewater: Klebsiella pneumoniae, Pseudomonas putrefaciens, Escherichia coli, gram-negative rod, and Pseudomonas species. The wastewater sample was plated, colonies of these five morphological types were subcultured, and the pure species were mixed in various percentages and dilutions in deionized water for a total of 120 samples. The results, shown in figure 10, illustrate the relationship between the chemiluminescence light unit and the microbial count by the spread plate method. It was found that the relationship is linear with a slope of one and that a luminol light unit is equivalent to 2×10^6 cells/ml, independent of bacteria species. Of particular interest is the measurement precision: the standard deviation for the measurement (0.17 σ) is comparable to the conventional plate count method (0.12 σ), which requires 10 to 15 times as long to perform.

The chemiluminescence biosensor was tested in the MIST from May 1976 to February 1977. During this time, chemiluminescence readings showed a strong relationship to turbidity (suspended solids) and a weak relationship to viability by plate count. These data confirm that the luminol reaction occurs with both viable and nonviable cells. Comparative microscopic and Coulter counts substantiated this conclusion. In addition, tests were conducted in which a culture of Escherichia coli was treated with ethylene oxide until there was no viable count, while leaving the cells intact. The luminol signal was unchanged by the treatment.

Bioluminescence system calibration was performed using the same five strains of bacteria from JSC wastewater by a procedure similar to that described for the chemiluminescence system. Six methods were investigated for determining ATP content of the samples: peak signal or area of signal with one, two, or three enzyme injections. It was found that the measurement error was reduced by half when evaluating the average peak signal from three injections. This measurement error (0.16 σ) compares favorably with the chemiluminescence sensor (0.17 σ) and the plate count method (0.12 σ).

The calibration test data in figure 11 show that cellular ATP content varies by as much as a factor of 10 between species. However, in their natural environment, it is not expected that the ratio of species would vary significantly or that the additional error introduced in calculating cell populations by assuming an average ATP/cell would be minimal. Calibration of the coliform detector was delayed until the start of the field testing and is addressed in the following section.

FIELD TESTING AND RESULTS: PHASE III

The WMS was operated at the SWTP from April 4 through June 3, 1977. Testing was aimed at demonstrating system operation and effectiveness in monitoring in-process fluid and effluent from an actual wastewater treatment facility. Fully automated operation over a sustained period with only routine manual adjustments was accomplished. Sensors were calibrated under software control and returned to service for continuous monitoring of the plant. Reports were generated on an hourly basis from data stored on disk.

System Operation

The system nominally ran 24 hours each day, Monday through Friday, for a total of more than 1000 hours of operation. Some system downtime was experienced because of power failures, sample pumping problems, and computer shutdowns. The trailer was manned during the day shift; however, system operation was computer-controlled except for short periods when individual sensors were manually standardized to check for proper functioning or when the computer system was down. The system design allows for manual operation during computer downtimes, although data recording is severely hampered.

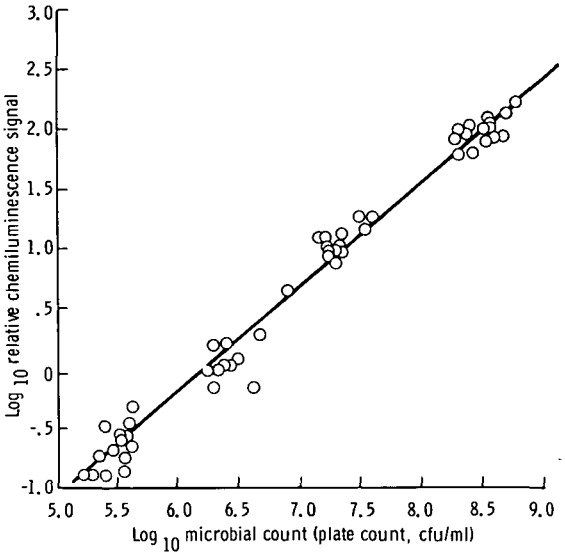


Figure 10.- Chemiluminescence signal as a function of plate count of bacteria isolated from JSC wastewater.

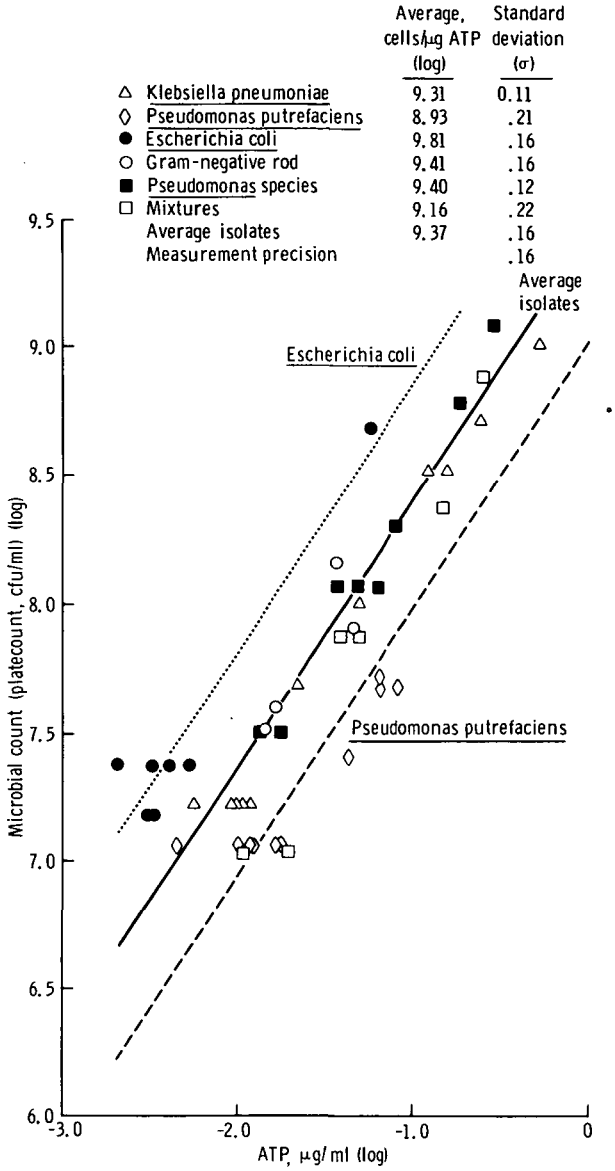


Figure 11.- Bioluminescence signal as a function of plate count of bacteria isolated from JSC wastewater.

Throughout the test period, effluent sensors monitored final plant effluent drawn from a sample point just above the weir. Multipoint sensors monitored final effluent, clarifier effluent (prechlorination), and mixed liquor. Lack of test time prevented sampling from other in-process sources. Multipoint sources were changed on the odd hours. Thus, each source was monitored a minimum of 2 consecutive hours to allow for sensor standardization/stabilization and at least 1 hour of data recording. To help maintain system cleanliness during this trial period, final effluent was sampled every other 2-hour period.

The effectiveness of the major elements of the system is discussed in the following sections.

Sample conditioning distribution system.- The sample conditioning/distribution system performed extremely well throughout the test period while pumping clarifier and final effluent; required maintenance was minimal. However, problems were encountered during sampling of mixed liquor. Because the test plant has no primary settling, large objects such as rags were frequently pulled into the system and almost immediately blocked the lines and filters. This problem was partially solved by placing a coarse screen over the intake pipe at the sampling point. With this modification, the system was able to supply unfiltered samples to the appropriate multipoint sensors with only periodic maintenance. Sensors requiring filtered samples from the mixed liquor could not be supplied for more than very short intervals because of filter clogging and resulting pressure losses.

Biosensors and bacteria concentrator.- The three biosensors were operated daily throughout the field test period. Luminescence determinations were made every 2 hours using 10-microliter samples for each sensor. Effluent samples were assayed during the odd hours and multipoint sources during the even hours. The coliform detectors were inoculated once each day. In addition to regular monitoring, assays were run to provide additional calibration information for each of the luminescence sensors and to begin calibration of the coliform detectors.

Calibration information for the luminescence sensors was determined by the same technique described in the Assembly and Checkout section. The JSC microbiology laboratory identified the following four predominant microbial species in sample water from the SWTP: Pseudomonas putrefaciens, Klebsiella pneumoniae, Bacillus pasteurii, and Bacillus sphaericus. Test data are summarized in table III with data on JSC wastewater given in parentheses. Insufficient assays were run to produce complete calibration curves.

The coliform detector cells were manually inoculated daily during the field test period. However, the first weeks of the test were spent on further development work on the sensor and significant data were not obtained until the sixth week. These data are shown in figures 12(a) and 12(b), which relate fecal and total coliform plate counts to detector lag-time measurements. Consistent results were obtained from prechlorinated samples as indicated by the narrow scatter of data. Results obtained on final effluent samples are more scattered. Only one sample from the mixed liquor tank was analyzed.

Samples were obtained directly from plant sources for inoculation in the coliform detector. These samples were analyzed by the JSC laboratory for total viable cells and all coliform cells. The laboratory results obtained were the basis of the coliform detector calibration. An analysis of samples was also performed by the Houston microbiology laboratory to cross-check results. The data are in general agreement, but the number of test samples analyzed by the Houston laboratory was insufficient to establish an independent calibration curve.

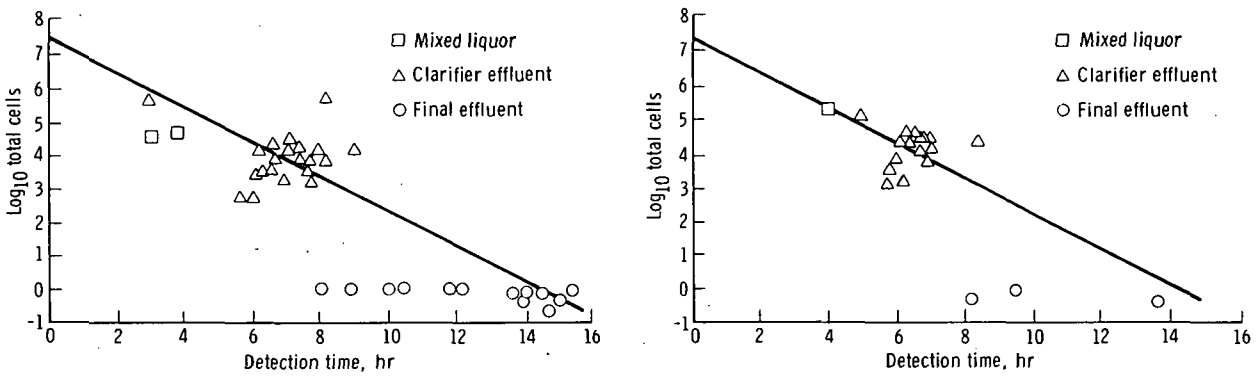
Calibration of the detector was not completed before termination of the field testing. Analysis of the available results, however, indicates that

TABLE III.- LUMINESCENCE BIOSENSOR CALIBRATION DATA,

CITY OF HOUSTON'S SOUTHWEST TREATMENT PLANT

Species	Bioluminescence, cells/μg ATP (a)	Chemiluminescence, (cells/ml)/ percent signal (a)	Plate count, cells/ml	
			Unchlorinated	Chlorinated
Pseudomonas putrefaciens	1.90×10^9 (0.85×10^9)	1.60×10^5 (1.60×10^5)	500	0
Klebsiella pneumoniae	5.00×10^9 (2.00×10^9)	1.90×10^6 (1.10×10^6)	40 000	0
Bacillus pasteurii	1.30×10^9	$.45 \times 10^5$	300	30
Bacillus sphaericus	$.67 \times 10^9$	$.41 \times 10^5$	2 000	10
Weighted average in unchlorinated effluent	3.70×10^9	5.40×10^5		
Weighted average in chlorinated effluent	1.05×10^9	-		

[a]JSC wastewater data are given in parentheses.



(a) Fecal coliform.

(b) Total coliform.

Figure 12.- Coliform calibration test results.

the best approach is to obtain a cell reaction in the 1 to 10 cell count region by serial dilution of mixed liquor samples or by using larger sample volumes of final effluent.

Bacteria concentrator performance data were calculated from chemiluminescence measurements. Forty-three measurements were made, with an arithmetic average of 67.5 percent recovery.

Commercial sensors.- All operable commercial sensors functioned on a continuous-flow basis, giving real-time data on the sample being monitored. A brief summary of individual sensor performance follows.

Ammonia analyzer: The performance of the ammonia analyzer was generally satisfactory. However, being a wet chemical analyzer intended for laboratory use, it contains several gravity drains and small fluid and air pumps that are potential problem areas in an on-line configuration. Particular problems included bubbles in the analyzer tubing, leakage of glued fittings, contamination of reagents, algae growth in drain lines, and formation of a brownish film on the optical cell. Frequent cleanings required at least 1 hour of downtime each day. The nitrite/nitrate analyzer, also a wet chemical laboratory-type instrument, experienced similar difficulties.

Chloride analyzer: Failure of a cassette pump forced this instrument to be shut down early in the test period. Very little data were accumulated, and the performance of the chloride analyzer could not be evaluated.

Chlorine analyzer: The chlorine analyzer performed very well. The one-a-day calibration was adequate, and the analyzer showed little drift over 24 hours.

Conductivity sensor: the only calibration required by the conductivity sensor was a check of the electronics. This check was performed manually once each week, and no change was noted throughout the test period. The sensor performed exceptionally well and did an excellent job of evaluating the operation of the reverse osmosis cartridges as well as routine sample monitoring.

Dissolved oxygen meter: The dissolved oxygen meter was not evaluated. A tear developed in the oxygen permeable membrane shortly after field testing began, and a new membrane could not be secured before testing ended.

Hardness analyzer: The analyzer itself functioned well; however, automatic standardization was often faulty and, as a result, a large amount of test data was lost.

pH analyzers: Both units were standardized daily and were found to drift very little in 24 hours. Some discrepancy was noted in the readings, but this was found to be caused by using standards having a lower temperature than the actual samples.

Sodium analyzer: Problems with the sodium analyzer centered around a faulty flowmeter on the analyzer that regulated the flow of sample or standard to the electrode well. Once this was corrected by a thorough cleaning, the instrument operated very well.

Temperature sensors: The calibration of the temperature sensors was manually checked twice during the field test. Both tests showed the readings to be within 1^{o} of the actual temperature, using a laboratory thermometer as the standard. Occasional erratic readings were noted when monitoring the same

sample with both sensors. This condition was found to be the result of a loose-fitting electronics module in one of the sensors and was quickly corrected.

Total organic carbon/total oxygen demand/total nitrogen: Although the total organic carbon (TOC) analyzer performed well, a large amount of TOC data was lost when either total oxygen demand (TOD) or total nitrogen (TN) units experienced problems that required taking the total analyzer system off-line. Some data from all three channels were also lost because of computer problems. The TOD failures were usually in the electronics. It was discovered near the end of the field test that the analyzer's output was not linear as had been thought. The TN analyzer experienced a variety of electronic and operational problems that hampered data recording throughout the test period. It should be noted, however, that this instrument is a "first of a kind" unit and has not been totally "debugged."

Turbidity: The Sigrist photometer was usually calibrated twice each week or whenever the standard was changed. It showed excellent stability and was practically trouble-free. At times, there was some difficulty in maintaining a good flow stream through the measuring head when the sample source was changed, but this was a minor annoyance rather than a serious problem. The unit gave an immediate and reliable response to changes in sample turbidity. The only basic problem with the Honeywell analyzer concerned the sample well. This well holds approximately 0.02 cubic meter (5 gallons) of sample through which the analyzer sends a light beam to be reflected from the bottom of the well back to a photocell in the analyzer head. This large amount of water caused a sluggish response to changes in the turbidity of the samples. It also allowed for errors in the readings due to the buildup of solids on the bottom of the sample well.

Most of the sensors were automatically standardized at least once each day by computer-controlled valve actuation and mode sequencing. In the early phase of testing, this standardization procedure began each day at 8 a.m. Because this interfered with "hands-on" operation (such as development work and routine maintenance), the time was changed to 10 p.m., when the system was unmanned. When WMS personnel arrived each morning, they requested from ADAM a printout of the previous night's standardization data for each sensor. If the values were satisfactory, normal operation was allowed to continue. If irregularities were found, a new system standardization was requested of the computer or individual sensors were manually standardized.

The time required for automatic standardization varies with the individual sensor. Turbidity and dissolved oxygen (DO) meters require only 1 to 2 minutes, while the wet chemistry sensors, such as those for ammonia and nitrate, require the longest period--2 hours 15 minutes. Each sensor was automatically placed back on-line as soon as its standardization was completed. In addition to this daily routine, multipoint sensors went through an automatic stabilization/standardization period each time the sample source changed. The TOC/TOD/TN sensors were restandardized, requiring 55 minutes off-line. Other sensors were put into a 25-minute delay period to allow them to stabilize before data recording was begun.

The overall performance of the automatic standardization system was good. The system proved to be capable of automatically standardizing the commercial sensors on a regularly scheduled basis, thus saving many hours of operator time.

Data acquisition system.- Instantaneous reports of data from the commercial sensors and system operating parameters were updated on the CRT each minute. At the end of each hour, a hard copy of the report was printed. In addition, a daily report and a summary of hourly averages was printed each day. More than 1000 of these various reports were generated, giving an hourly record of plant operation over a 2-month period. Biosensor data reports were generated separately on an "as needed" basis.

A typical instantaneous report is shown in table IV. The first set of data contains the values for the multipoint source (3-HOUSTON SWTP MIXED LIQUOR) and the second set of data contains the values for the effluent source (6-HOUSTON SWTP EFFLUENT).

A typical daily summary report is shown in table V. It includes the number of data points, the daily average, and the instantaneous and hourly peak values and the time of day each occurred for each source of water sampled throughout the day. The effluent sensors are the first data set printed, followed by each multipoint sample source. Data plotting capability, as shown in figure 13, is also available as a system feature.

The luminescence reports provided the results of the bioluminescence and chemiluminescence analyses every 2 hours. A typical luminescence report is given in table VI. The voltage values and the final cell count are shown for the ATP (bioluminescence) and CHEM (chemiluminescence) sensors for both concentrated and unconcentrated samples. The sample source used for each analysis is indicated by the "/N" numbers printed after the voltage values. The less-than sign (<) indicates that the results are less than the indicated value because the difference between the voltage readings was less than 0.10 volt.

Coliform results required 3 to 14 hours after cell inoculation for determinations. The results were available for individual cells after the minimum voltage value and the 200-millivolt time was determined. A typical coliform report is shown in table VII.

As is common to new software systems, a significant number of "startup" problems were experienced. Considerable historical data were destroyed on the disk files as a result of conflicts between data and input/output (I/O) operations. Near the end of the field test period, it was found that many of these system errors were being caused by intermittent hardware malfunctions that resulted from an overheating problem on both EVE memory boards. These problems were corrected, and satisfactory operation of the computer/data system was realized. However, the current EVE program is I/O bound; i.e., calculations are delayed and interrupted by I/O requests because the system resources are overloaded. This overload caused delays in report generation and

TABLE IV.- TYPICAL INSTANTANEOUS DATA REPORT

TIME - 144:14:00:00 SAMPLING POINT: 3-HOUSTON SWTP MIXED LIQUOR

CHA NO.	SENSOR	UNITS	STATUS	INST VALUE	*** AVERAGES *** 15 MIN	30 MIN	1 HR	RUNNING
6.	TURBIDITY-SG	MG/L		428.0	393.3	363.4	389.8	296.5
7.	DIS OXYGEN-DS	MG/L		0.6	0.6	0.6	0.7	1.2
8.	TOT OXY DEM	MG/L	VARI	1766.	2449.	2494.	2526.	2545.
9.	TOT ORG CARB	MG/L	LMIT	752.0	589.7	598.6	596.3	599.6
10.	AMMONIA	MG/L	ZERO	24.0	0.0	0.0	0.0	0.1
11.	NITRATE	MG/L	ZERO	114.0	0.0	0.0	0.0	0.0
12.	PH-GL	PH	OUT					
13.	CHLORIDE	MG/L	OUT					
14.	CONDUCTIVITY	MMMHO/CM		774.8	774.4	775.8	778.7	767.3
15.	TEMPERATURE#1	DEG F		80.8	80.5	80.7	81.6	82.3
21.	MULTIPOINT	PSIA		38.6	38.4	38.6	38.6	38.4
27.	D. I. PRESS	PSIA	OUT					
28.	TOT NITROGEN	MG/L		129.0	152.9	155.9	161.4	155.3

TIME - 144:14:00:00 SAMPLING POINT: 6-HOUSTON SWTP EFFLUENT

CHA NO.	SENSOR	UNITS	STATUS	INST VALUE	*** AVERAGES *** 15 MIN	30 MIN	1 HR	RUNNING
0.	COLIFORM	VOLTS		-2.730				
2.	BIOLUM	VOLTS		-7.730				
3.	AIR COMP	PSIA		99.9	95.8	96.1	95.3	95.4
5.	RES CHLORINE	MG/L		3.5	3.5	3.5	3.6	2.8
16.	HARDNESS	MG/L		125.0	128.0	127.9	126.1	105.3
17.	SODIUM	MG/L	OUT					
18.	TURBIDITY-HW	FTU		6.7	6.7	6.7	6.7	6.4
19.	DIS OXYGEN-HW	MG/L	OUT					
20.	TEMPERATURE#2	DEG F		83.6	83.4	83.3	83.2	81.5
23.	EFFLUENT	PSIA		58.1	57.1	57.4	58.9	63.7
30.	PH-OR	PH	OUT					
39.	CHEMILUM	VOLTS		-3.810				

hampered software development work on the system. The Data General NOVA 1200 used for EVE contains the maximum amount of memory available--32 768 words--and cannot be expanded.

Comparison With Laboratory Analyses

During the field testing, daily composite samples and a large number of discrete samples were taken for physical and chemical laboratory analyses. These samples were collected at a point immediately outside the trailer. Early in the field testing, simultaneous samples were also taken directly from the appropriate tanks to determine the effect, if any, of the polyvinyl-chloride (PVC) pipes carrying sample water to the trailer. In general, analyses on samples from the two locations were comparable. Some scatter was found in nitrogen (ammonia and nitrate) and microbial determinations, but no significant trends were noted.

TABLE V.- TYPICAL DAILY DATA REPORT

DAILY REPORT FOR: 6-HOUSTON SWTP EFFLUENT 5/25/77 00:00:00

CHA NO.	SENSOR	UNITS	DATA POINTS	DAILY AVERAGE	INSTANTANEOUS PEAK VALUE	INSTANTANEOUS PEAK TIME	HOURLY PEAK VALUE	HOURLY PEAK TIME
0.	COLIFORM	VOLTS	247	1.724	3.550	11: 4	3.247	11: 0
2.	BIOLUM	VOLTS	OUT					
3.	AIR COMP	PSIA	1323	95.2	100.0	0: 0	96.0	10: 0
5.	RES CHLORINE	MG/L	1270	2.7	4.4	7:47	4.1	8: 0
16.	HARDNESS	MG/L	1261	105.6	133.0	12:30	131.9	12: 0
17.	SODIUM	MG/L	OUT					
18.	TURBIDITY-HW	FTU	1321	6.7	7.9	0: 0	7.7	0: 0
19.	DIS OXYGEN-HW	MG/L	OUT					
20.	TEMPERATURE#2	DEG F	1323	82.2	86.4	21:47	84.0	18: 0
23.	EFFLUENT	PSIA	1323	62.7	74.2	14:16	66.0	5: 0
38.	PH-OR	PH	OUT					
39.	CHEMILUM	VOLTS	228	5.424	9.995	15:44	8.097	4: 0

DAILY REPORT FOR: 3-HOUSTON SWTP MIXED LIQUOR 5/25/77 00:00:00

CHA NO.	SENSOR	UNITS	DATA POINTS	DAILY AVERAGE	INSTANTANEOUS PEAK VALUE	INSTANTANEOUS PEAK TIME	HOURLY PEAK VALUE	HOURLY PEAK TIME
6.	TURBIDITY-SG	MG/L	325	289.5	592.0	17:24	463.0	12: 0
7.	DIS OXYGEN-DS	MG/L	334	1.5	10.9	17:40	3.7	18: 0
8.	TOT OXY DEM	MG/L	205	1994.	3248.	17: 6	2820.	13: 0
9.	TOT ORG CARB	MG/L	217	486.5	794.0	14:16	614.0	13: 0
10.	AMMONIA	MG/L	22	0.1	0.3	12:40	0.1	13: 0
11.	NITRATE	MG/L	OUT					
12.	PH-GL	PH	OUT					
13.	CHLORIDE	MG/L	OUT					
14.	CONDUCTIVITY	MMMHO/CM	334	776.1	836.6	18: 0	798.4	18: 0
15.	TEMPERATURE#1	DEG F	334	82.4	97.6	15:13	85.5	13: 0
21.	MULTIPOINT	PSIA	334	38.0	41.1	17:59	38.6	14: 0
27.	D. I. PRESS	PSIA	OUT					
28.	TOT NITROGEN	MG/L	204	97.4	200.0	13:56	161.4	14: 0

DAILY REPORT FOR: 4-HOU SWTP CLARIFIER EFFLUENT 5/25/77 00:00:00

CHA NO.	SENSOR	UNITS	DATA POINTS	DAILY AVERAGE	INSTANTANEOUS PEAK VALUE	INSTANTANEOUS PEAK TIME	HOURLY PEAK VALUE	HOURLY PEAK TIME
6.	TURBIDITY-SG	MG/L	247	19.4	544.0	10:58	93.2	11: 0
7.	DIS OXYGEN-DS	MG/L	395	5.0	7.4	1: 1	5.4	6: 0
8.	TOT OXY DEM	MG/L	132	45.	152.	10:54	119.	11: 0
9.	TOT ORG CARB	MG/L	132	6.2	10.0	6:41	8.4	2: 0
10.	AMMONIA	MG/L	392	4.5	7.8	2: 1	7.3	2: 0
11.	NITRATE	MG/L	OUT					
12.	PH-GL	PH	OUT					
13.	CHLORIDE	MG/L	OUT					
14.	CONDUCTIVITY	MMMHO/CM	395	779.9	818.2	21: 4	807.6	22: 0
15.	TEMPERATURE#1	DEG F	395	80.0	83.6	1:26	83.3	2: 0
21.	MULTIPOINT	PSIA	395	39.4	44.7	10:55	40.0	6: 0
27.	D. I. PRESS	PSIA	OUT					
28.	TOT NITROGEN	MG/L	15	17.5	57.0	21:59	48.2	22: 0

DAILY REPORT FOR: 6-HOUSTON SWTP EFFLUENT 5/25/77 00:00:00

CHA NO.	SENSOR	UNITS	DATA POINTS	DAILY AVERAGE	INSTANTANEOUS PEAK VALUE	INSTANTANEOUS PEAK TIME	HOURLY PEAK VALUE	HOURLY PEAK TIME
6.	TURBIDITY-SG	MG/L	299	17.0	544.0	10:58	93.2	11: 0
7.	DIS OXYGEN-DS	MG/L	494	5.1	7.4	18: 1	5.6	20: 0
8.	TOT OXY DEM	MG/L	169	117.	2400.	18: 3	331.	19: 0
9.	TOT ORG CARB	MG/L	154	22.9	590.0	18: 3	123.1	19: 0
10.	AMMONIA	MG/L	473	3.7	7.8	2: 1	7.3	2: 0
11.	NITRATE	MG/L	OUT					
12.	PH-GL	PH	OUT					
13.	CHLORIDE	MG/L	OUT					
14.	CONDUCTIVITY	MMMHO/CM	496	786.2	841.2	18: 6	825.7	20: 0
15.	TEMPERATURE#1	DEG F	496	80.3	83.6	1:26	83.3	2: 0
21.	MULTIPOINT	PSIA	496	38.9	44.7	10:55	40.0	6: 0
27.	D. I. PRESS	PSIA	OUT					
28.	TOT NITROGEN	MG/L	41	43.5	200.0	18:45	56.8	19: 0

TABLE VI.- TYPICAL LUMINESCENCE DATA REPORT

LUMINESENCE REPORT: 5/24/77

		* * * * * * * * * * * * HOUR OF DAY * * * * * * * * * * * *					
	UNITS	1	2	3	4	5	6
ATP							
FILT	VOLTS	0.020		0.012		0.014	
STAND	VOLTS	0.026		0.011		0.025	
UNCON	VOLTS	0.024/4		0.020/6		0.011/4	
	C/ML	< 2.5+ 8		< 2.5+ 8		< 2.1+ 8	
CONC	VOLTS	0.024/4		0.028/6		0.030/4	
	C/ML	< 1.7+ 6		< 2.6+ 6		2.3+ 6	
CHEM							
BLANK	VOLTS	-3.881		-3.872		-3.916	
FILT	VOLTS	-3.910		-3.893		-3.911	
UNCON	VOLTS	-3.742/6		-3.784/6		-3.754/6	
	C/ML	6.7+ 6		4.4+ 6		6.3+ 6	
CONC	VOLTS	7.808/4		6.265/6		2.849/4	
	C/ML	0.0+ 0		0.0+ 0		0.0+ 0	
UNCON	VOLTS	-3.871/4		-3.553/6		-3.483/4	
	C/ML	1.5+ 6		1.4+ 7		1.7+ 7	

TABLE VII.- TYPICAL COLIFORM DATA REPORT

COLIFORM REPORT: 5/13/77

	* * * * * * * * * * * * SENSOR * * * * * * * * * * * *							
	1	2	3	4	5	6	7	8
INOCULATION TIME	12:26	12:30	15:37	15:37	15:38	15:38	12:32	12:28
MINIMUM VOLTS								
TIME	13:35	13:35	17:44	16:37	29:31	16:39	13:35	16:39
VALUE	0	38	39	-22	32	38	88	38
200MV TIME	17:53	19:24	19:14	18:32	29:38	19:29	19:35	29:38
REACTION TIME	5:27	6:54	3:37	2:55	14: 0	3:51	7: 3	17:10
CELLS/ML								
TOTAL					1.0+ 0	1.2+ 5	3.0+ 3	1.0+ 0
FECAL	1.9+ 4	3.5+ 3	1.6+ 5	3.5+ 5				

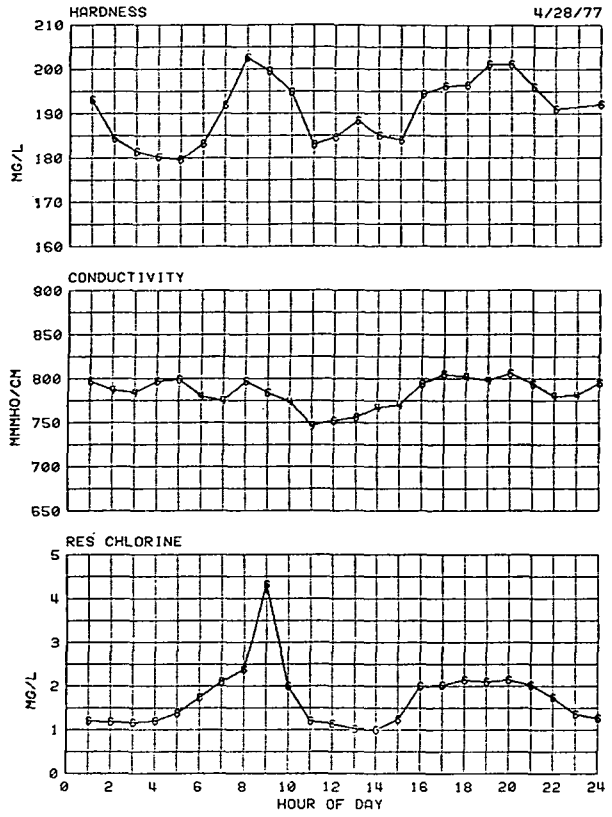
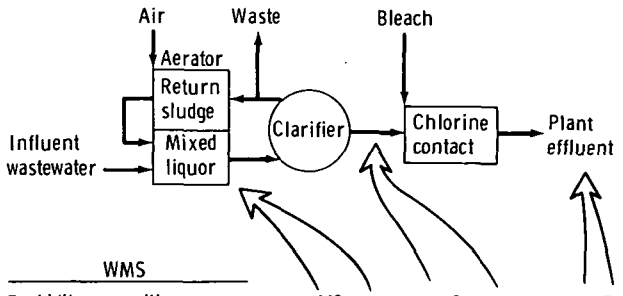


Figure 13.- Data plot from field test.



WMS			
Turbidity, ppm silica	460	8	7
TOC, ppm	800	50	50
TOD, ppm	3000	80	80
TN, ppm	120	10	10
Total residual chlorine, ppm			2
Total microbes, cells/ml	$2x10^9$	$3x10^6$	$3x10^6$
ATP, cells/ml	$4x10^9$	$3x10^5$	$1x10^5$
Total coliform, cells/ml	$1x10^5$	$2x10^3$	1
Fecal coliform, cells/ml	$5x10^4$	$4x10^2$	<0.1
Laboratory			
Total suspended solids, ppm	5000	5	5
BOD, ppm		3	3
COD, ppm	2200	10	10
Coulter count, cells/ml		$3x10^6$	$3x10^6$
Plate count, cells/ml	$7x10^6$	$6x10^4$	100
Total coliform, cells/ml	$3x10^5$	$4x10^3$	1
Fecal coliform, cells/ml	$1x10^4$	$4x10^2$	None

Figure 14.- Typical data: Houston Southwest Wastewater Treatment Plant.

Residual chlorine was determined at the field test site. Samples for other analyses were collected in glass containers and transported immediately to the appropriate laboratories. Time-critical analyses (such as ammonia) were performed the same day. Laboratory microbial determination by both laboratories were made on the same water used for manual sample injection in the coliform detector cells. The samples were packed in ice or kept refrigerated until used by the laboratories. More than 1100 laboratory analyses were performed. Typical WMS and laboratory data from field testing are shown in figure 14.

Despite the large volume of data collected, comparison of WMS readings with conventional laboratory analyses was of limited success. Few of the chemical laboratory analyses performed could be directly related to WMS data. Both the laboratory and the WMS had equipment for determining ammonia, chloride, hardness, residual chlorine, fecal coliform, and total coliform. However, the chloride and hardness sensors in the WMS were inoperable during the

field test and the ammonia sensor was only marginally reliable. Of the remaining three parameters, residual chlorine showed reasonably good agreement, and the total and fecal coliform detector demonstrated good correlation with the standard laboratory plate counts.

Of those determinations not directly comparable, it was anticipated that some type of correlation would be found between such organic loading parameters as TOC and BOD, total suspended solids and turbidity, BOD and turbidity, and TOC and chemical oxygen demand (COD). Statistical analysis of the data for these sets of parameters was performed using linear regression by the least-squares method. In each case, however, the correlation coefficients for the resulting equations showed that the correlation between the two parameters was poor.

Agreement between bacteria plate counts and the number of bacterial cells based on the ATP measured by the bioluminescence sensor was poor. Ten times more ATP was measured in unchlorinated effluent and 1000 times more in final effluent than could be accounted for by plate counts of organisms. With mixed liquor samples, the ATP measurement agreed with published data; i.e., approximately 1 g/ml (ref. 10). However, again the ATP content and plate count measurements disagreed, with ATP indicating 10^9 cells/ml and the plate counts 10^6 cells/ml. Previous determinations on treated wastewater samples at the MIST facility had shown a similar pronounced difference--as much as 3 orders of magnitude--between cell populations indicated by ATP content and by plate counts. However, oceanographic studies have shown that the ATP levels found at various ocean depths indicate bacteria populations between 50 and 2000 times those found by plating techniques (ref. 12).

Excessive ATP cannot be attributed to variations in bacterial species distributions because the levels measured at the field test site and at the MIST facility were approximately the same. However, a possible explanation for the difference is that the laboratory-prepared species used to calibrate the sensor are, in fact, allergic to a laboratory environment and thus exhibit different characteristics from those in a natural environment. Or, bacteria in the final effluent tank might show increased ATP if the cells are metabolizing, but not dividing, in the presence of the chlorine. Further work is necessary to explain the apparently excessive ATP in natural waters. The in situ ATP measurement may well provide a more comprehensive description of the total biological material that is maintaining a life balance with its environment than the plate counts performed under laboratory conditions.

An examination of ATP reaction inhibition by substances in the wastewater was performed; the results are given in table VIII. It was found that the reaction was unaffected by bleach disinfectant. Tris reagent was used as the standard reference for these tests. As a result of these findings, the assay procedure was changed to standardize with filtrate to account for the inhibiting effect.

Also investigated was the effect of bacteria loss due to filtering of the effluent and the multipoint samples. Results indicated no significant loss due to filtering the effluent samples. Losses due to filtering

TABLE VIII.- ATP REACTION INHIBITION BY WASTEWATER[a]

Medium	Signal	Percent loss
Tris	8.2 units	Reference
Unchlorinated effluent	2.5 units	70
Chlorinated effluent	2.5 units	70

[a]0.1-μg ATP/ml sample.

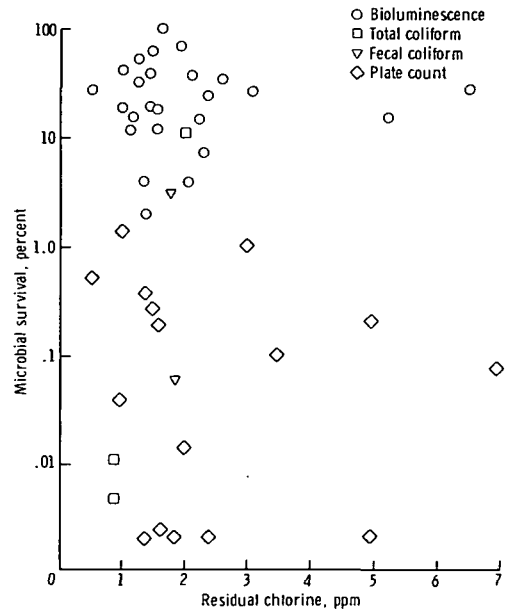


Figure 15.- Microbial survival as a function of residual chlorine level.

multipoint samples were found to be significant during analysis of mixed liquor and return sludge, as noted in table IX, and filtration of the multipoint samples for the biosensor was discontinued.

It would appear that the chemiluminescence measurements of both live and dead cells should be closely related to Coulter counts, which are counts of total particles in solution. This assumption was found to be valid in the two instances in which Coulter counts were made on fresh samples. Because of laboratory equipment problems, other Coulter counts could not be made until the samples had aged to the extent that the data were considered invalid.

Effect of Plant Operating Conditions on Water Quality

Although the test period was not long enough to fully characterize the plant by investigating the effect of various plant operating conditions (such as flow rate, retention time, and biomass level) on all water quality parameters, a significant amount of data was accumulated on two aspects of plant operation: diurnal flow-rate cycle and residual chlorine level.

The SWTP shows a typical diurnal flow-rate cycle for a domestic wastewater treatment plant with two peak flow periods--one occurring at midmorning and the other shortly before midnight. Flow rate decreases to a minimum in the early morning hours (1 to 6 a.m.). The following general trends in data were noted.

TABLE IX.- BACTERIA LOSS DUE TO FILTERING

Bacteria	Unfiltered (a)	Filtered (5 μm) (a)	Percent loss
Mixed liquor	320	1.4	99.56
Return sludge	620	2.8	99.5
Escherichia coli	170	150	11.76

[a]Arbitrary units.

1. Turbidity and conductivity were affected very little by the diurnal cycle. Periods of heavy rainfall did greatly increase turbidity, however, and generally lessened effluent quality.

2. TOC and TOD showed wide daily ranges and frequently peaked when flow rate was low, particularly at night.

3. Ammonia, nitrite/nitrate, and TN data showed considerable fluctuations that appeared to be unrelated to flow rate.

4. Residual chlorine levels frequently rose at periods of low flow (discussed in greater detail in the following paragraphs).

5. Bacterial content and DO were remarkably stable both through the diurnal flow-rate cycle and from day to day throughout the test period.

Two significant aspects concerning residual chlorine levels were demonstrated during field testing. First, early test data showed that the residual chlorine level at the test facility was not consistently maintained at the normal operational level of 1 to 2 ppm. After corrective procedures were instituted, residual chlorine levels became more consistent. However, keeping the level relatively constant required frequent manual residual chlorine monitoring, flow-rate determinations, and manipulation of the hypochlorite addition valves by plant operators.

Second, a series of tests was run on the final effluent to determine microbial survival as a function of residual chlorine in the effluent. Test data, shown in figure 15, were derived by measuring organisms (total, total coliform, and fecal coliform) with plate counts, coliform detector, and bioluminescence measurements for prechlorinated samples and final effluent containing increasing concentrations of residual chlorine. The following conclusions can be drawn from these tests.

1. Increasing the chlorine concentration more than 1 to 2 ppm showed no improvement in elimination of organisms detected by any of the methods.

2. An average microbial survival rate of 50 percent was observed when measuring organisms in terms of ATP.

3. By comparison, microbial survival was 1 percent or less according to laboratory plate counts.

4. The coliform detector and plate counts both confirmed that total and fecal coliform organisms were effectively and consistently eliminated by chlorine disinfection.

DISCUSSION

The water monitoring system resulting from this project represents the most advanced system existing today for on-line real-time water quality monitoring. Although the system was and continues to be developmental, the significance of the project and what it represents to the field of water technology is worthy of further mention here. The project has undertaken a bold approach toward bringing the field of water monitoring into the age of automation in several important respects. The most important of these advances is the capability to perform real-time on-line biological monitoring. This area is one that has long been of interest to spacecraft life support system designers who have the responsibility for crew health. The WMS represents the first serious effort to advance this technology into the community. However, the long period of "status quo" in the field of water technology has yet to be overcome. There are those who maintain that rapid monitoring is not required and therefore not worth the expense. The price of that attitude will be a continued reliance on slow laboratory methods at the possible sacrifice of community health and a clean environment. With the advent of the WMS, it is hoped that additional advancements and implementation of WMS-type systems in existing and future facilities will be considered. Unique advances posed by the NASA system are as follows.

1. The first totally real-time systems approach to monitoring water quality

2. An automatic sample conditioning/distribution system that provides constant sample flow from any of several preselected sources

3. Conventional water quality sensors adapted for continuous-flow sampling and automatic standardization

4. Computer-controlled operation including automatic standardization, valve sequencing, and report generation

Although testing of the field demonstration unit reported herein was not sufficiently long or sufficiently comprehensive to adequately verify all specific system capabilities, it did demonstrate two major points: (1) the system has great potential for future water monitoring and/or process control application, and (2) the WMS represents a vast improvement over conventional (grab sample) water monitoring techniques. Future applications of the system offer two significant and unique contributions: real-time assurance of water quality and monetary savings by using continuous feedback for process control.

With the WMS, commercial sensor data were available on a minute-by-minute basis, and microbiological data were available in no more than 12 hours, with routine luminescence measurements requiring only 10 to 15 minutes. Comparable physical and chemical measurements from the laboratory required a minimum of 1 day. Standard laboratory microbial determinations required a minimum of 2 to 4 days. This time differential is the most significant feature of the WMS. It is this time factor that would allow the WMS to give immediate verification of water quality, thus protecting receiving bodies against pollution or making reuse of treated wastewater acceptable. In addition, it is this time factor that would allow the WMS to be used effectively in process control operations.

Process control of a wastewater treatment facility by the WMS presents several ways of reducing operating costs. One of these ways--optimum control of hypochlorite addition--was demonstrated at the field test site. As mentioned earlier, real-time monitoring of the residual chlorine level at the SWTP helped plant operators to achieve more consistent levels of chlorine than had previously been maintained. Because levels usually had varied on the high side during periods of reduced flow, the effect was to reduce the amount of hypochlorite disinfectant used by the plant. It is expected that even greater reductions could be made if the WMS actually controlled hypochlorite addition using real-time feedback of residual chlorine levels.

At the SWTP plant, approximately $9000 to $10 000 each month (roughly $300/day) is spent for chlorine disinfectant to treat the 0.095- to 0.114-m^3/day (25- to 30-MGD) flow of the plant. Maintaining a 1- to 2-ppm residual chlorine level requires the addition of about 18 kilograms (40 pounds) of available chlorine for each 3785 cubic meters (1 million gallons) of wastewater at a cost of $0.2675/0.45 kilogram ($0.2675/pound). At these rates, operating a plant of this size for half the time at even 1 ppm above the 1- to 2-ppm desired range would cost an average of $1000/month in additional disinfectant requirements. And, as the microbiological tests demonstrated, this increased level of residual chlorine offers no corresponding increase in the quality of the effluent and might even be detrimental to the effluent-receiving body. Thus, it is essentially lost money flowing over the weir.

A second and potentially more important area of savings in operating cost would be WMS control of the many large compressors that provide millions of cubic feet of air each minute for aeration at a typical activated sludge treatment plant. Energy costs to run these compressors represent one of the largest expenditures in the operation of a wastewater treatment plant.

Optimization of the amount of air fed to aeration tanks by real-time monitoring of biomass system parameters and effluent quality could produce substantial reductions in energy requirements.

To meet its full potential, the present WMS needs further development to eliminate several operational problem areas and to reduce maintenance requirements. Several of the commercial sensors are inadequate for on-line monitoring and others need further adaptations to improve their reliability and durability. The biosensors exhibited significant potential but need further calibration and test investigation; simplified operating procedures requiring less operator attention; greater reliability; and, in the case of the bioluminescence sensor, reduced operation costs and a determination of the significance of an ATP measurement in relation to standard methods of water quality monitoring.

In the DAS, field testing showed that the storage capability of the report generation computer was not adequate to meet present needs and certainly not adequate for anticipated future needs. The computer memory was overloaded, and the mainframe is not capable of expansion to add the required memory. For future use, additional memory and disk storage will be required to meet new demands imposed by increased biosensor processing and control; integration of new sensors, some requiring special batch processing; and increased complexity of operator interfaces and communications including data plotting.

As an outgrowth of this future development and upgrading, the WMS must demonstrate that it can meet water monitoring requirements not fulfilled by conventional laboratory analysis. This will no doubt involve not only modifications to the WMS itself, including some new sensors, but also a gradual change in the type of data (parameters) considered as the standard for water quality determination; e.g., replacement of the standard BOD test by an automated on-line analysis providing data that gives similar information on water quality. (Such an instrument and parameter has yet to be discovered). For these changes in standard methods to occur, the types of analyses included in the WMS must be carefully chosen and thoroughly tested in actual wastewater treatment environments to fully characterize the type of information derived and its relationship to, and comprehensiveness in, defining total water quality.

RECOMMENDATIONS

The design, development, and testing of the field demonstration unit during Phases II and III of the WMS project produced an abundance of operational data and experience. This, in turn, has resulted in a number of recommendations for future evaluations, modifications, and testing of the system.

1. Because of prior commitments, the initial field trial in Houston was a very limited trial-operation experience intended primarily to provide an initial exposure of the system to the local Houston and Texas community. An extended operational period of one or more sites is required to permit the WMS to undergo extensive, long-duration testing in a wastewater treatment

facility. This will also allow a stabilized operational laboratory routine to evolve. With the deployment of the WMS at the Santa Clara Valley Water District facility in Palo Alto, California, the opportunity for such an extended operational evaluation exists.

2. A deliberated evaluation procedure should be established for the testing. This procedure should involve both NASA and the Santa Clara Valley Water District (or other future users) so that a user perspective may be factored into the operation. Laboratory support from the users commensurate with this task should be required.

3. Because of the significance of this system development, additional evaluation and demonstration sites should be found, after the Santa Clara Valley evaluation is completed, to provide a wide system exposure for the technology. Additional or alternative federal or state funding support for such continued operations is considered to be warranted and should be sought.

4. In light of the flexibility of the system, consideration should be given to operating and evaluating the WMS in process control applications and for process streams other than wastewater.

5. Following this extensive test period, a complete review of system capabilities should be made, with particular consideration to current water quality analyses required by regulatory agencies and to those analyses envisioned as standard methods in the future.

6. Development work on a method to expand the capability of the chemiluminescence biosensor to enable differentiation between viable and nonviable cells should be continued. This method (ref. 13) involves bubbling carbon monoxide through a sample. Cell metabolism of the carbon monoxide results in a loss of luminol signal from viable bacteria. The difference between the luminol response from untreated and treated samples is then attributed to the viable cells. This technique works in the laboratory but has not yet been adapted to a continuous-flow system for field evaluation.

7. Wherever possible, the commercial analyzers evaluated in the WMS should be of the solid-state electrode variety as opposed to wet chemistry types. Efforts should be undertaken to replace current wet chemistry sensors with new instrument methods where available to reduce the number of man-hours required for routine maintenance.

8. The report generation computer should be replaced with a more powerful unit. The next generation Data General computer system, a NOVA 3D, would provide sufficient memory (65 536 words) for present needs and is compatible with the current peripheral equipment and interface controllers.

CONCLUDING REMARKS

The NASA Lyndon B. Johnson Space Center water monitor system project was concluded in June 1977 with the completion of Phases II and III as described in this report. Extensive testing and some further development of the system are still required. However, with completion of the Houston field test and appropriate documentation, most of the WMS project objectives have been met. They are as follows.

1. Real-time micro-organism sensor technology has been developed to the point where three types of biosensors--measuring total viable bacteria, total live and dead bacteria, and total and fecal coliform--are functioning in automated on-line configurations.

2. An automated WMS for wastewater or water treatment application has been developed that incorporates advanced real-time micro-organism detection and conventional and advanced sensor technology for nonbiological parameters.

3. The WMS field demonstration unit has been put through a short field demonstration test at a municipal wastewater treatment facility.

4. This report and other pertinent WMS papers will be distributed to federal, state, and local agencies in an effort to disseminate the technology acquired. In addition, model specification packages of the field demonstration unit with system drawings and plumbing and electrical diagrams are available for distribution.

Lyndon B. Johnson Space Center
National Aeronautics and Space Administration
Houston, Texas, May 2, 1979
644-02-00-00-72

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APPENDIX A

DESCRIPTION OF COMMERCIAL SENSORS

AMMONIA

A Delta Scientific Model 8119 Ammonia Analyzer continuously measures ammonia in sample water by spectrophotometric analysis. The intensity of the blue color developed by the reaction of ammonia with phenol and hypochlorite in an alkaline medium is proportional to the concentration of ammonia in the sample. The analyzer has a minimum sensitivity of 0 to 1 ppm ammonia, with no upper limit.

CHLORIDE

A Great Lakes Instruments (GLI) Chloride Ion Probe measures chloride by a GLI-patented Differential Electrode Technique. The GLI approach compares an Orion Model 94-17 solid-state chloride electrode to a standard electrode containing a chemical chloride ion standard. The probe is housed in a 3.8-centimeter (1.5-inch polyvinylchloride (PVC) tee that has been modified to reduce interior volume and provide a rapid flow past the probe. Before reaching the probe, the sample is mixed 50 to 1 with an ionic strength/pH adjuster. The probe has a sensitivity range of 10×10^5 to 5×10^5 moles/liter of chloride ion. It is relatively free from interferences except for sulfur, bromide, iodide, and cyanide.

CONDUCTIVITY

The sensor used to measure the ionic content of the water sample is a Beckman Type R15 Solu-Bridge Conductivity Indicator with a temperature-compensated epoxy flow-through cell, type CEL-VDJ4-KF. The cell constant is 4.0, enabling measurements in the range of 0 to 2000 μmhos/cm.

DISSOLVED OXYGEN

A Delta Scientific Series 8310 Automatic Analyzer continuously measures dissolved oxygn in a sample stream with a tank-mounted DO probe. The patented probe consists of gold and silver electrodes mounted in a PVC body. A Teflon membrane forms an oxygen-permeable barrier between the water being tested and the electrolyte in the probe. A voltage is applied across the electrode and, as oxygen passes through the membrane, it produces an electrical current proportional to its concentration. DO readings in milligrams per liter and parts per million in the ranges of 0 to 2, 0 to 10, or 0 to 20 are displayed.

The Honeywell Model 551011-00-01 dissolved oxygen sensor is a polarographic transducer consisting of a gold/silver/platinum alloy cathode and a silver/silver chloride anode immersed in an electrolyte cell. Its operation is similar to that of the Delta Scientific instrument.

HARDNESS

An Orion Model 1132 Hardness Analyzer continuously monitors the sample stream for hardness, a measure of the calcium and magnesium ions in water. The technique used is proprietary to Orion Instrument Company. However, in general terms, it involves the chelation of all divalent ions by a complexing agent, followed by the addition of a "substitution" ion that selectively releases calcium ions. A "tag" ion is added at molar concentrations 100 times greater than the maximum possible ionic strength. A reference electrode is selective to this tag ion, and the sensing electrode is selective to the substitution ion. The electrodes are connected to the analyzer's electronics system, which gives a direct hardness reading on a four-cycle logarithmic scale calibrated to read from 0.1 to 1000 mg/liter.

NITRATE

A Delta Scientific Model 8183-153105-002XX1 Nitrate Analyzer continuously measures nitrate/nitrite concentrations in the sample stream by spectrophotometric analysis. Nitrates are reduced to nitrites in a cadmium reducing column. The nitrites are then reacted with sulfanilamide and N-1 Napthyl-Ethylenediamine Hydrochloride (NEDA) in an acid solution to form the azo dye. The color intensity developed is a measure of the nitrate plus nitrite concentration in the sample. The concentration of nitrites may be determined separately by bypassing the cadmium column. Nitrate/nitrite concentrations above 0.4 ppm are too dark for useful discrimination. Therefore, dilution of the sample is required for most measurements.

ACIDITY/ALKALINITY (pH)

The Great Lakes Instruments (GLI) Model 70 Analyzer measures pH for in-process and pollution applications. The probe uses the GLI Differential Electrode Technique to compare a pH electrode to a standard electrode containing a chemical pH standard. The probe is housed in a PVC tee (as described for the chloride ion probe) to increase the flow rate by the probe.

An Orion Model 401 specific ion meter provides a direct readout of pH using an Orion 91-01 pH electrode and a Beckman 19033 Lazaran process electrode. The pH electrode has a range of 2 to 11 pH units. Both electrodes are mounted in small tanks that provide continuous monitoring capability.

RESIDUAL CHLORINE

An Orion Model 1125 Chloride Analyzer measures the residual chlorine in continuous samples by the potentiometric method. It operates on the principle that chlorine will liberate free iodine from potassium iodide solutions when the pH is 8 or lower. The sample is mixed with a reagent and pumped through a reaction heater and a constant temperature analysis chamber. In the chamber, the mixture passes between a sodium electrode and a redox electrode. The electrodes are connected to the analyzer's electronics system, which gives a direct chlorine concentration reading on a four-cycle logarithmic scale calibrated to read from 0.1 to 1000 mg/liter.

SODIUM

The Beckman 19402 Model 9415 Sodium Ion Analyzer determines the concentration of sodium in a sample stream by measuring the potential between a Beckman 633951 Sodium Ion Electrode and a Beckman 19604 reference electrode. The electrode potential is directly proportional to the logarithm of the active sodium concentration. The response of the sodium electrode can be affected by several other monovalent cations. Usually, hydrogen is the only interfering ion encountered. To eliminate this interference, all solutions are pH adjusted with ammonia to suppress the hydrogen ion concentration. Temperature control is provided by a heat exchanger located upstream of the electrode flow chamber.

TEMPERATURE

Two Action Pac Resistance Thermal Detectors (RTD) are used to measure sample temperatures. The RTD probe detects changes in potential between two electrodes as the temperature changes and converts this to a 0- to 5-volt signal. The probe is sensitive to 0.1° and reads from 0 to 200° F.

TOTAL ORGANIC CARBON/TOTAL OXYGEN DEMAND

The Astro Ecology Corporation analyzer simultaneously determines TOD and either total carbon or TOC on aqueous samples containing solid particulates of as much as 2000 micrometers in diameter. Dual measurement ranges of 0 to 10 ppm or 0 to 500 ppm of carbon and 0 to 100 ppm or 0 to 1000 ppm of oxygen are available.

A 50-ml/min sample is continuously pumped into a mixing chamber and gas-scrubber assembly where it is mixed with hydrochloric acid to reduce the pH to 3. Inorganic carbon is converted to carbon dioxide, which is vented to an airstream. A portion of the scrubbed carbonate-free sample (4 ml/min) is pumped into a combustion chamber and combined with a metered airstream

(79-percent oxygen). The air provides the oxygen for combustion. The sample remains in the chamber at 1123.15 K (850° C) long enough for full combustion to occur. The products of combustion and excess air leave the reactor and enter a water-cooled liquid/gas separation assembly that removes considerable vapors. The remaining gases are routed to an infrared analyzer where the amount of carbon dioxide is measured and converted to units of TOC.

The TOD analyzer determines oxygen demand for hydrogen, nitrogen, sulfur, and carbon compounds found in the sample water. It receives the noncondensable gases from the TOC analyzer following the carbon dioxide analysis and passes them through a solid electrolyte oxygen detector. The oxygen depletion, based on the amount of air fed to the reactor, is measured and translated into units of TOD.

TOTAL NITROGEN

The IBC/Berkeley Nitrogen Analyzer receives the noncondensable combustion gases from the TOD analyzer and determines the concentration of nitric oxide by measurement of the potential between two electrodes. During the combustion at 1123.15 K (850° C), all nitrogen in the sample is converted to nitric oxide; thus, a total nitrogen reading is provided by the instrument in the range of 10 to 10 000 ppm nitrogen.

TURBIDITY

A Sigrist Photometer Turbidimeter Model UP 52-TJ determines the turbidity of a continuous sample stream by comparison with a nephelometric standard. Model UP 52-TJ has four measuring attachments of different ranges. Two of the units use falling-stream flow cells with ranges of 2 to 1000 and 2000 to 15 000 Jackson turbidity units (JTU), one uses a surface scatter-flow cell with a range of 5 to 100 JTU, and one uses a splash-flow cell with a range of 0.5 to 20 JTU. All of them use a dual-beam optical measure bridge. In the DAS, the units of measurement are converted to milligrams per liter of silicon dioxide.

The Honeywell Model 551201-02-01 turbidity sensor has an optical head and a sampling tank for on-line analysis. A beam of light is focused by a lens system down into a falling sample. A photocell detects the light reflected by particles in suspension and supplies a proportional millivoltage to the signal conditioner. The sensor has a nominal range of 0 to 25 Formazin turbidity units (FTU).

APPENDIX B

BIOSENSOR DEVELOPMENT SUMMARY

Development of the NASA biosensors into on-line real-time instruments involved extensive research and experimentation. The tests performed and the techniques developed during this period are summarized in this appendix.

CHEMILUMINESCENCE

Chemiluminescence detection, based on measurement of light emitted by the catalytic action of bacterial porphyrins on a luminol/hydrogen peroxide mixture, is complicated by the fact that a light response is also produced by several interfering agents such as metallic ions and extracellular porphyrins. Two techniques were developed to eliminate these interferences and give a measurement specific for bacteria.

1. Pretreatment with hydrogen peroxide. Organic interference is eliminated by incubating the sample for 2 minutes with a dilute hydrogen peroxide solution. This solution does not affect the bacterial porphyrins but rapidly reacts with and deactivates soluable porphyrins such as catalase and hemoglobin. The bacterial porphyrins can then be reacted with luminol after the cells have been ruptured by sodium hydroxide in the luminol reagent. The data in table B-I show that this procedure is effective in eliminating more than 90 percent of this type of interference.

2. Reaction rate resolution. Inorganic interference is eliminated by taking advantage of the difference in light emission rates as shown in figure B-1. When the light measurement is made 10 seconds after mixing, light from inorganic interferences has dissipated while bacteria-produced light remains.

Table B-II illustrates the performance of the two interference elimination methods in various kinds of water. This indicates that 99 percent of the interference found in tapwater was eliminated with no degradation in response to bacteria.

In wastewater, interference was variable. Although the signal for bacteria-free wastewater was usually less than 1 light unit, it occasionally increased significantly and twice produced a saturated signal. On both occasions, the water was a putrid yellowish color and killed the bacteria in the pilot plant biological treatment unit. The contaminant was not identified but was assumed to be a solvent from a manufacturing, cleaning, or photographic process. These experiences demonstrated the necessity of accounting for background light (noise). Tests showed that the bacteria signal was additive to the background signal. Consequently, the bacterial content of a sample is evaluated in terms of net signal (signal minus noise; e.g., sample minus bacteria-free sample signal).

TABLE B-I.- EFFECT OF 0.5 PERCENT HYDROGEN PEROXIDE PRETREATMENT ON SOME LUMINOL OXIDIZING AGENTS

Oxidizing agent	Reduction of signal, percent
Catalase	94
Hemoglobin	95
Extracted bacterial porphyrins	97
Potassium ferricyanide	50
Cobalt(ous) chloride	20
Ferrous sulfate	0

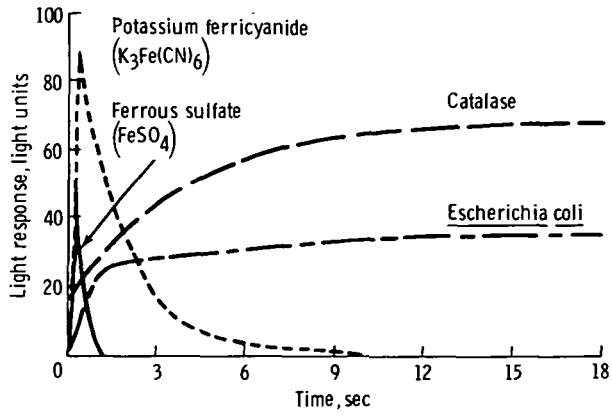

Figure B-1.- Reaction rate curves for various luminol oxidizing agents.

TABLE B-II.- PERFORMANCE OF INTERFERENCE ELIMINATION METHODS

Sample	Conductivity, μmho	Untreated sample, light units	Pretreatment only, light units	Reaction rate only, light units	Both	
					Light units	Percent reduction
Tapwater	1000	41	3.5	2.4	0.7	99
Reverse osmosis effluent (tapwater supply)	100	17	2.1	.8	.3	99
Deionized water	.1	.5	.4	.2	.2	100
Escherichia coli in deionized water	.1	70	65	88	73	0

BIOLUMINESCENCE

The bioluminescence sensor detects the ATP in living cells by measuring the light emitted in a biochemical reaction with purified luciferase enzyme extracted from firefly tails. Earlier laboratory work used commercially purified luciferin-luciferase. In an attempt to defray operating costs, a less expensive supply of luciferase was sought. Luciferase can be extracted from desiccated firefly lanterns. With appropriate purification steps and the addition of synthetic luciferin, the resulting enzyme exhibits better activity and costs only one-tenth that of the commercial product.

The procedure developed for obtaining highly purified, less expensive luciferase is outlined as follows.

A. Preparation of acetone powder

1. Grind firefly lanterns (Sigma Chemical Company, Worthington, California) and a small amount of sand with mortar and pestle, keeping the mixture cold with liquid nitrogen or an acetone/dry ice mixture.

2. Add cold acetone (277.15 K (4° C))--at least 100 milliliters per 5 grams of tails--and wait 10 minutes to dissolve lipids.

3. Filter solution through a Buchner funnel and wash with cold acetone.

4. Completely dry powder and store at 253.15 K (-20° C).

B. Preparation of crude luciferase-luciferin extract

1. Add 10 milliliters of cold 0.05M Tris, pH 7.75 with 1×10^{-3}M Cleland's reagent per gram of acetone powder. Mix gently at 283.15 K (10° C) for 30 minutes.

2. Centrifuge the solution at 10 000 units relative centrifugal force with reference to gravity for 10 minutes and collect the supernatant; discard precipitate.

C. Purification of luciferase

1. Bring the supernatant from step B-2 to 30 percent ammonium sulfate $(NH_4)_2SO_4$ at room temperature and discard precipitate.

2. Then, bring solution to 70 percent $(NH_4)_2SO_4$ and collect precipitate.

3. Wash precipitate in 70 percent $(NH_4)_2SO_4$ at pH 7.75.

4. Dissolve washed pellet in Tris buffer: 0.05M, pH 7.75, 1×10^{-3}M Cleland's reagent, 1×10^{-2}M magnesium sulfate ($MgSO_4$) (minimum volume 2.5 milliliters per gram of acetone powder). Centrifuge to clarify. Apply enzyme to Sephadex G-200 column and elute with same Tris buffer. Assay fractions by mixing small portion 1:1 with luciferin in Tris buffer.

5. Pool fractions with high activity and low inherent light. (One gram of acetone powder should produce about 20 milliliters). Dilute pool with Tris buffer to desired activity level. Add 0.1 milligram luciferin for each milliliter of diluted fraction pool. Aliquot and lyophilize enzyme. Store desiccated at 253.15 K (-20° C).

6. Rehydrate in 0.05M Tris pH 7.75, or 0.20M Tris pH 8.2, or 0.25M Tris pH 8.2.

COLIFORM DETECTION

The detection of coliform organisms using electrochemical methods to measure hydrogen was demonstrated in the microbiology laboratories at the NASA Langley Research Center and at JSC. The response of the platinum/calomel electrode in the coliform sensor to serial dilution of Escherichia coli is illustrated in figure B-2. A period of low electrical activity following inoculation, defined as the lag period, is followed by a rapid rise in the electrode signal as hydrogen is detected. Each tenfold increase in the inoculum reduces the hydrogen detection time by approximately 1 hour. The exact time of detection may be defined as the minimum voltage point or as a given rise in voltage, the latter being preferred if some electrode instability is experienced.

Figure B-3 gives the results of 50 tests performed in the laboratory showing the relationship between total cells in the sample and detection time. These data indicate a measurement standard deviation of 0.89 (log total cells).

Automating the coliform detector required developing a cleanup procedure that would eliminate all viable organisms from the previous analysis. Heat by autoclaving is the normal laboratory bacteriostat technique. A major constraint on the sensor is that the calomel/platinum electrode deteriorates in an environment above 353.15 K (80° C). Bacteriostat of the electrode was successfully accomplished in the laboratory by soaking in a water bath at 358.15 K (85° C) for one-half hour.

Other problem areas resolved during development of the automated sensor were (1) selection of sodium hypochlorite over 3 percent hydrogen peroxide because of adverse effects of the peroxide on the electrode, and (2) elimination of sources of metallic ions, also because of their effect on electrode performance. The sensor system components in direct contact with the incubation cell or connecting fluid lines were fabricated from plastic or glass.

Automatic injection of samples was discontinued for the present because of the inability to provide effective bacteriostat of the sample delivery line. Detector cells are inoculated by syringe injection of the sample through a septum.

BACTERIA CONCENTRATOR

To provide concentrated samples for the biosensors, the following requirements were established for a bacteria concentration system: (1) the system must provide up to 1000-fold concentration, (2) at least 40 milliliters of concentrate must be produced, and (3) the system must be compatible with automation.

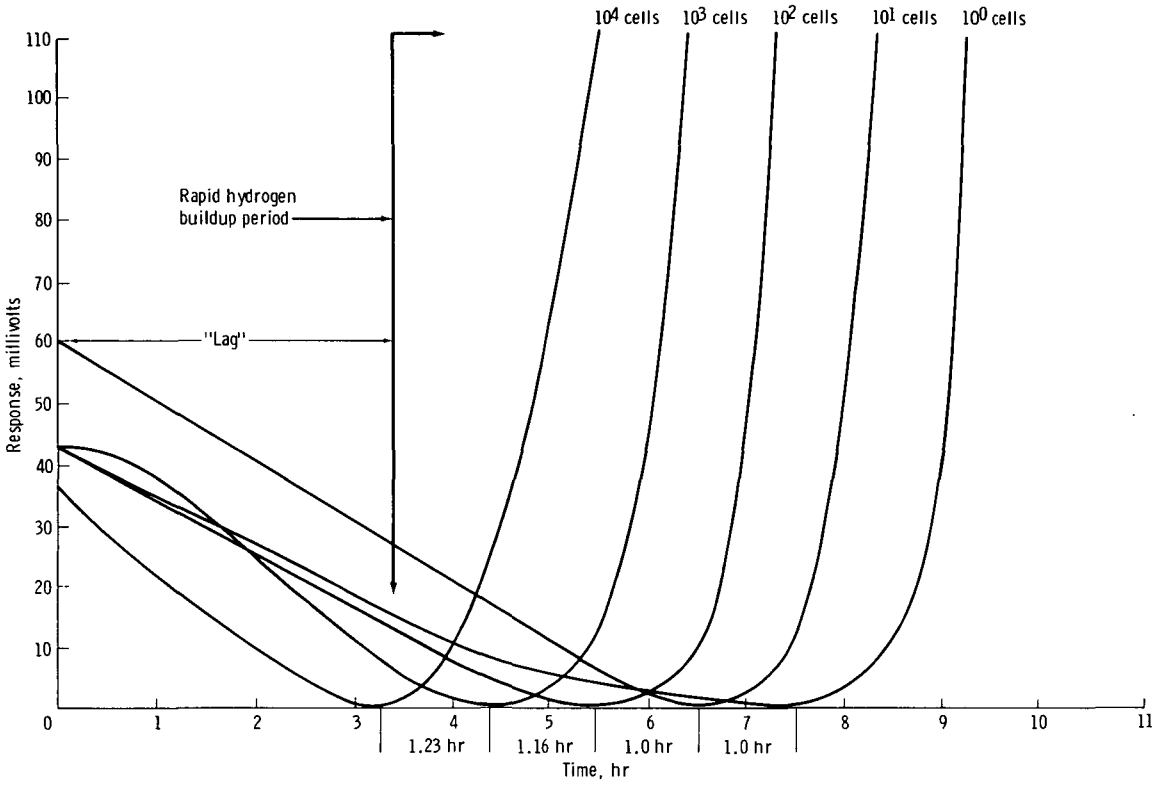

Figure B-2.- Response of platinum/calomel combination electrode to E. coli metabolic hydrogen with serial dilution inoculum.

The devices tested to determine their characteristics as bacteria concentrators are listed in table B-III. Test data are given in table B-IV, and the results are summarized as follows.

1. The best performance was obtained with a hollow-fiber device in a backwash mode.

2. For this application, flat surface membrane devices were undependable.

3. A centrifuge provides adequate recovery (approximately 75 percent) but at low concentration rates. The centrifuge was not readily adaptable to the requirement for automation and was not further pursued.

4. The cyclone provided practically no recovery.

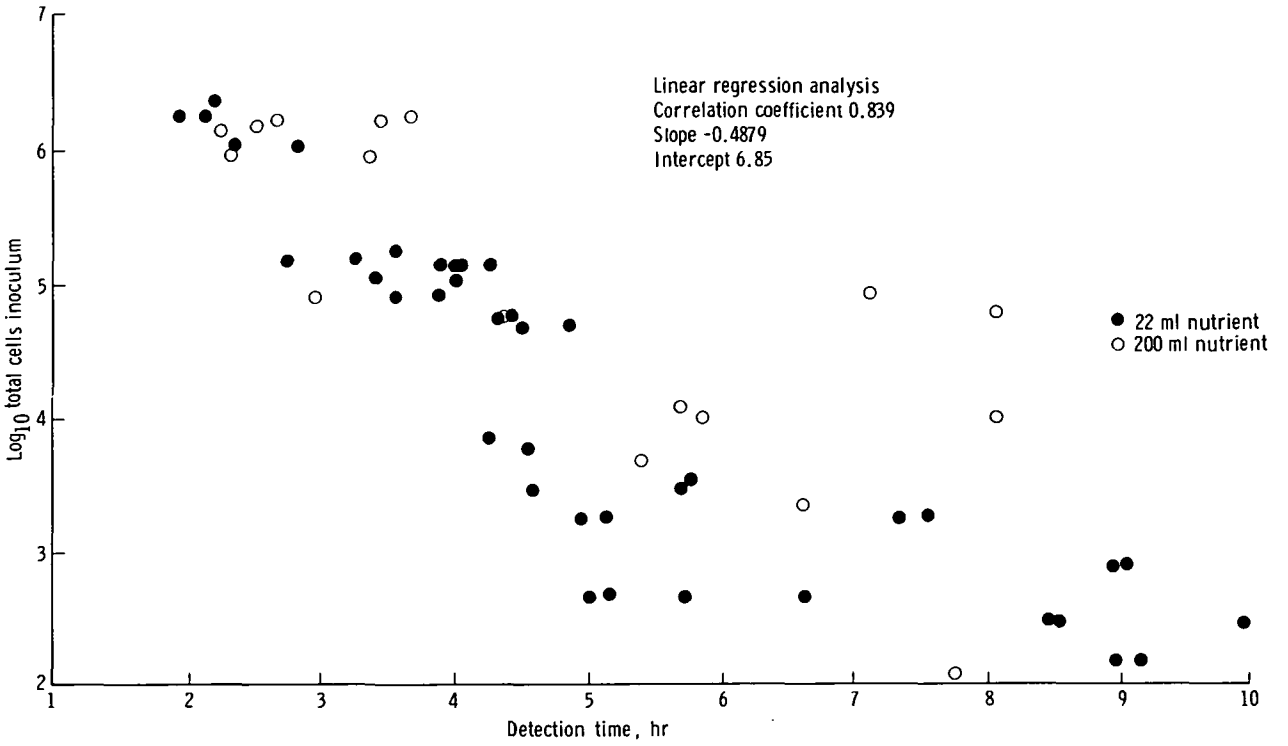

Figure B-3.- Plot of data showing the relationship between log total cell inoculum and detection time at 30 millivolts above the minimum voltage.

TABLE B-III.- TEST DEVICES FOR BACTERIA CONCENTRATORS

Device	Specifications	Manufacturer
	Flat-surface membrane filters	
High-volume molecular filtration cell	Pellicon cassettes, type PSSP - 10 000 molecular weight (MW) cutoff, type PTCK - 100 000 MW cutoff, cellulose membrane	Millipore Corporation
Uni-Pore dual-membrane stirred cell	Uni-Pore polycarbonate membrane filter disks, 90-mm diameter, 0.2 and 0.4 μm	Bio-Rad Laboratories
Sartorius Ultrafiltration System	Uni-Pore polycarbonate filters, 1 to 15 stacked plates, 170-cm^2 filter area per plate, 0.2 and 0.4 μm	Sartorius Corporation
	Hollow-fiber membrane filters	
Bio-Fiber Miniplant	10 000-cm^2 filter area, 30 000 MW cutoff, cellulose acetate fibers	Bio-Rad Laboratories
Diaflow hollow fibers	0.093 and 0.930 m^2 (1 and 10 ft^2) filter areas, 50 000 MW cutoff, synthetic polymer fibers	Amicon Corporation
	Centrifugation	
Sorvall SS-3 Superspeed centrifuge	KSB continuous-flow system, 0 to 20 000 rpm, 50-ml collection tubes, fixed-angle rotor	DuPont Instruments
Cyclone	Plexiglass, 5.7- to 0.7-cm inside-diameter cone, 15.1-cm long, 0.038 m^3/min (10 gal/min), 689 476 N/m^2 (100 psi)	NASA contractor (NAS 9-11984)

TABLE B-IV.- BACTERIA CONCENTRATOR TEST RESULTS

(a) Flat membranes

Concentrator (a)	Test conditions/results					
	Procedure	Sampling rate, ml/min	Filtrate rate, ml/min	Concentration, times/min	Recovery, percent	Recirculation rate, ml/min
High-volume molecular filtration cell	Continuous	100 to 1000	100 to 800	2 to 10	0 to 90	
	Recirculation	100 to 500		1 to 5	0 to 50	100 to 500
	Backwash	200 to 1000		1 to 5	0 to 90	
Sartorius Ultrafiltration System	Recirculation and backwash	20 to 100		0.1 to 0.5	10 to 100	100 to 500
Uni-Pore dual-membrane stirred cell	Collection/ stabilization	500 to 1000		10 to 20	<50	

[a]Samples: bacteria seeded deionized water.

TABLE B-IV.- CONTINUED

(b) Hollow-fiber membranes

Concentrator (a)	Test conditions/results				
	Procedure	Sampling rate, ml/min	Concentration	Recovery, percent	Recirculation rate, ml/min
Bio-Fiber 80 miniplant	Backwash only	100 to 400	15 to 250 (1 to 4 times/min)	64 ± 23	400 to 1000
	Recirculation	100 to 400	15 to 250 (1 to 4 times/min)	42 ± 17	400 to 1000
	Backwash, refilter, backwash	100 to 400	15 to 250 (1 to 4 times/min)	81 ± 13	400 to 1000
	Backwash, refilter, backwash, flush, backwash	100 to 400	15 to 250 (1 to 4 times/min)	83 ± 10	400 to 1000
Diaflow hollow fibers (1000-cm^2 filter area)	Backwash	60 to 100	15 to 60 (1 to 2 times/min)	95 ± 5	300
Diaflow hollow fibers (10 000-cm^2 filter area)	Backwash only	500 to 1000	20 to 600 (5 to 20 times/min)	53 ± 21	
	Backwash, refilter, backwash	500 to 1000	20 to 600 (5 to 20 times/min)	83 ± 14	
	Backwash, refilter backwash, flush, backwash	500 to 1000	20 to 600 (5 to 20 times/min)	88 ± 12	

[a]Samples: bacteria seeded deionized water and treated wastewater.

(c) Centrifuge

Concentrator	Test conditions/results				
	Sampling rate, ml/min	Concentration rate, times/min	Speed, rpm	Acceleration	Recovery, percent
Centrifuge	30 to 60	0.6 to 1.2	10 000	11 400g	45 to 71
	30 to 60	0.6 to 1.2	12 500	17 800g	45 to 82
	30 to 60	0.6 to 1.2	15 000	25 600g	68 to 89

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16. Abstract

A project was conducted to test a water quality monitoring system with the objective of on-line and real-time operation similar to the function in a spacecraft. The design goal was to develop a system with the capability to determine conformance to future high effluent quality standards and to increase the potential for reclamation and reuse of water. Although all system capabilities were not verified in the initial field trial, fully automated operation over a sustained period with only routine manual adjustments was accomplished. Two major points were demonstrated: (1) the water monitor system has great potential in water monitoring and/or process control applications; and (2) the water monitor system represents a vast improvement over conventional (grab sample) water monitoring techniques.

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